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Study of factors prevailing for the selection of mycorrhizal strains in ash pond

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PROJECT COMPLETION REPORT

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I – GENERAL

REFERENCE NO: 2109 - 1

TITLE OF THE PROJECT: **Study of factors prevailing for the selection of mycorrhizal strains in ash pond**

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SUMMARY

In this recently concluded project, extremely useful results were obtained which can form a strong basis for using this knowledge and the material generated through intensive collaboration, towards the reclamation of ash ponds. An in-depth understanding of the basis of strain selection, adaptation of mycorrhizal strains to a stressed environment, mass production and the resulting biochemical and chemical expressions of root-fungus interaction was obtained. The collaboration is continuing and several crucial experiments are planned based on the results of this project which will bring us closer to practical reality.

OBJECTIVES

- Strain collection, identification, isolation and classification inside and outside the ash pond.
- Attempts to obtain EM strains better adaptable to ash ponds.
- Strain selection under nursery conditions.
- Large-scale production of mycorrhizal inocula for nurseries and mycorrhizal seedlings for plantation.
- Investigation of the mechanisms of adaptation to an ash pond environment.
- Investigation of the root-fungus signalling in an ash pond environment.

II – WORK REPORT

Contents

Chapter 1
Introduction .... 1 – 2

Chapter 2
Task 1 - Strain collection, identification, isolation and classification inside and outside the ash pond .... 3 – 7

Chapter 3
Task 2 – Attempts to obtain EM strains better adaptable to ash pond .... 8 – 84

Chapter 4
Task 3 - Strain collection in nursery condition .... 85 – 104

Chapter 5
Task 4 - Large-scale production of mycorrhizal inoculum for nurseries and mycorrhizal seedlings for plantation .... 105 – 129

Chapter 6
Task 5 – Mechanism of adaptation to ash pond environment .... 130 – 139

Chapter 7
Task 6 – Investigation of the root-fungus signalling in an ash pond environment 140

Chapter 8
References .... 141 – 144

Chapter 9
Publications .... 145 – 146

Annexures

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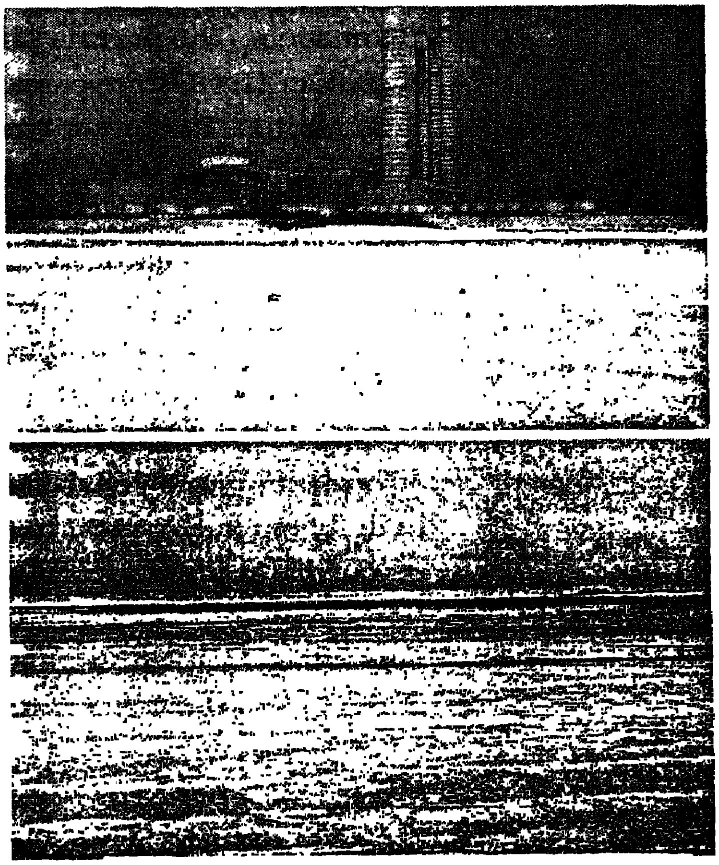
1

Introduction

Coal based thermal power plants currently generate about 70% of the electricity in India. The amount of ash generated is about 100 million tonnes per annum blocking 26,000 hectares of land nationwide. The coal ash produced in a power station as a result of a combustion of pulverized coal consists of bottom ash, which settles at the bottom of the furnace, and fly ash which is carried away by the flue gases and collected by electrostatic precipitators (ESP's). The prevailing disposal methods cause ecological disturbances and ash particles remain suspended in the air affecting plants, animals and human populations. An effective method of preventing ash particles from being suspended in the air is necessary. Maintaining green cover in and around ash dykes after they are abandoned is one such vital solution.



Fly ash dumps at Korba, Chattisgarh, Super Thermal Power Station

Attempts have been made to cover with vegetation, otherwise unpleasant and unproductive coal ash heaps around thermal power plants. Coal ash is rich in heavy metals, toxic to many plants, and possibly to useful microorganisms as well. It is also poor in nutrients. The chemical degradation of heavy metal ions present in coal ash is not a practical choice, so a biological mode could be attempted by applying mycorrhiza, beneficial fungi.

The programme aimed at developing an in-depth understanding of the interactions between heavy metal toxicity, and the nutritional properties of fly ash system. Parameters such as tolerance of the mycorrhizal fungi, but also for the host plants, nutritional status of organisms involved, soil properties, the levels and duration of exposures, the availability of metals and the specific behaviour will be studied. The results so obtained will form the basis of selecting candidates among plants and mycorrhiza for future reclamation efforts.

Mycorrhizal applications for land reclamation

Mycorrhiza are highly evolved, mutualistic associations between soil fungus and plant roots. The partners in these associations are members of the Fungus Kingdom (Basidiomycetes, Ascomycetes, and Zygomycetes) and most vascular land plants. Two main types of mycorrhiza have been distinguished: ectomycorrhiza and vesicular arbuscular mycorrhiza (VAM). In the ectomycorrhiza, the fungus grows intercellularly in the cortex of the plant root but never intracellularly. In VAM, the fungus grows both inter-and intra-cellularly forming the cortical cells specific fungal structures – vesicles and arbuscules – within and hence their name. They (VAM) colonize the plant root system and penetrate the root tissues to exchange nutrients with the host. This group of soil symbiotic microorganisms has already proved to be beneficial in afforestation and rehabilitation efforts. In the soil these fungi are able to thrive in deficient substrates. In substrates where phosphorous availability is low, mycorrhizal fungi are able to solublize the insoluble form of nutrients and make them available to the plants. Mycorrhiza are reported to sustain adverse effects because of high soil temperature, improve plant water relations and increase stomatal conductance besides being able to prevent soil-borne diseases.

Mycorrhizal infections can act as filter systems for coal ash overburdened sites. This will reduce the heavy metal content of the leachate and therefore the possibility of ground water contamination. The mycelium of mycorrhiza can spread to areas beyond the rhizosphere of the plant and access nutrients from the non-available pool. Plant infected with mycorrhiza have an increased cell wall surface because fungal mycelia inside and outside the roots, which are available for metal absorption. Although the exact process of heavy metal tolerance by mycorrhizal fungi is not very clear, two mechanisms may be involved: microorganisms can achieve resistance to heavy metals by 'avoidance' when the organism can restrict metal uptake or by 'tolerance' when the organism survives in presence of high internal metal concentrations etc. The first mechanism involves reduced uptake or increased efflux, formation of complexes outside the cell and organic acid release. In the second situation metals are cheleted intracellularly through the synthesis of ligands such as metalothioneins polyphosphates and/or compartmentation within the vacuoles.

2 Task 1 – Strain collection, identification, isolation and classification inside and outside the ash pond

TERI - India

Collection of EM and AM fungi from soils contaminated with industrial effluents and fly ash

Collections were made from the following sites in India.

1. VAM Organics, Gajrola - U.P.

Gajrola is situated 65 km north of Delhi. The site is about 1 acre in area with vegetation comprising grasses, herbs such as *Mazus* sp and *Parthenium* sp and members of *Euphorbiaceae*. The soil type was clay loamy.

2. Okhla Industrial Area (OIA) - Delhi

Okhla is about 13 km from TERI and lies in a semi arid zone. There are many industries discharging toxic effluents located there. Common vegetation includes grasses and herbs such as *Ricinus* sp, *Parthenium* sp, *Raphanus* sp, *Chenopodium album*. The soil was clay loamy.

3. Dadri Thermal Power Station (DTPS) – U.P.

This site, located in Uttar Pradesh is about 60 km from Delhi. The area is sparsely populated with *Thuja, Raphanus* and grasses.

4. Indraprastha Thermal Power Station, Delhi

It is situated 8 km from TERI. Vegetation included *Solanum esculatum, Raphanus* and grasses.

5. Korba Super Thermal Power Station (KSTPS) - Chattisgarh

This site is located in Chattisgarh, India, about 1246 km from Delhi. The vegetation consists of teak, sal and eucalyptus. The *Eucalyptus* plantation at KSTPS, Korba (BCPP) had a large population of *Pisolithus tinctorious*.

The following parameters were analysed in the samples (Table 2.1. 2.2)

Table 2.1 Bio-chemical parameters in the soil of industrial sites

SITES	*Avg. microbial count*	*VAM% Colonization*	*Mean number of spores of VAM/g of soil*	*OC (%)*	*Available P (ppm)*	*Total N (%)*	*EC (ds/m)*	*pH (1 25: soil.H_2O)*
VAM Organics Gajrola								
Sample 1	0.19×10^5	Nil	0.16±0.032	0.18±0.02	4.79±1.59	0.16±0 007	0 41	7.9
Sample 2	0.191×10^5	Nil	1.4±0.1	1.97±0.08	30.62±3.43	0.2±0.007	0 55	7 08
Sample 3	0.262×10^5	1.49	2.95±0.05	2.99±0.13	14.52±1.9	0.32±0.007	0.54	7 18
Sample 4	$.0.143 \times 10^5$	1.7	2.55±0.15	1.84±0.11	16.25±2.8	0.72±0.0107	1.98	7.5
Sample 5	0.178×10^5	0.68	2±0.4	1.83±0.12	20.08±2.3	1.35±0.007	2 54	7 25
Sample 6	0.282×10^5	0.018	2.82±0.046	1.16±0.08	5.28±2.1	0.44±0.0055	0 69	7 55
Okhla Industrial area								
Sample1	0.146×10^5	Nil	0.2±0.1	1.37±0.09	22.19±9.64	0.33±0.008	1 46	6 95
Sample2	0.187×10^5	Nil	0.2	0.12±0.03	24.37±1.06	0.52±0.066	1 92	7 13
Sample3	0.136×10^5	Nil	Nil	3.72±0.13	26.82±1.23	0.85±0 17	2 4	6 62
Sample 4	0.231×10^5	Nil	0.076	0.35	33.3±3.84	1.09±0.022	3 35	6.8
Sample5	0.305×10^5	Nil	0.173	2.31±0.16	35.32±3.91	0.82±0.011	3 92	6.8
Sample 6	0.272×10^5	Nil	0.3	1.67±0.09	35.29±2.86	1.08±0.028	2 02	6.9
Sample 7	0.187×10^5	Nil	0.2	0.71±0.06	15.77±4.26	0.3±0.014	0 25	7.6
Sample8	0.215×10^5	Nil	Nil	0.46±0.07	12.08±2.48	0 15±0.007	1	7 24
Sample9	0.225×10^5	Nil	0.1	1.95±0.09	32.85±3.79	0.15±0.014	1 87	6.9
Sample10	0.142×10^5	Nil	0.4	0.64±0.07	27.99±7.53	0 28±0.018	0 97	7.52
Sample 11	0.097×10^5	Nil	0.2	1.66±0.13	24.97±1.57	2.12±0 595	1 56	7.21
DTPS Dadri								
Sample 1	0.268×10^5	0.5	2.5±0.5	0.15±0.05	8.98±2.26	0.14±0 018	1 35	9.25
Sample 2	0.171×10^5	0.11	2.466±0.45	0.22±0.04	10.72±1.44	0.2±0.018	0 45	8 98
Sample3	0.092×10^5	Nil	0.4±0.26	0.27±0.05	11.83±3.33	0.14±0.032	3 61	9.28
Sample4	0.250×10^5	Nil	0.6±0.05	0.81±0.09	4.97±1.22	0.25±0 01	0 51	7.85
Sample 5	0.148×10^5	Nil	0	0.16±0.07	8.9±1.43	0.16±0.01	0 63	7.45
Sample 6	0.179×10^5	1.142	2.9±0.17	0.28±0.06	5.1±1.28	0.2±0.014	0.64	7 72
Indraprastha Thermal power station								
Sample 1	0.135×10^5	Nil	0.8±0.3	0.33±0.03	8.15±1.78	0.29±0 02	1 53	7.5
Sample 2	0.141×10^5	Nil	3.7±0.7	0.73±0.11	9.16±2.63	0.43±0 01	0 96	7 51
Sample3	0.235×10^5	Nil	2.3±0.5	0.65±0.04	20.43±6.97	0 29±0.007	2 07	7 15
Sample4	0.256×10^5	Nil	0.9±0.26	1.04±0.04	18.6±2.03	1.33±0.02	3 17	7 09
Sample 5	0.313×10^5	Nil	2.1±0.3	0.82±0.09	9.72±1.2	0.18±0.007	2 91	7 4
KSTPS, Korba								
Sample1	0.18×10^4	-	-	18.40	0.231	1.60	0 11	5 50
Sample2	1.83×10^4	-	-	10.00	0.329	2.35	0.03	5.91
Sample3	1.28×10^4	-	-	26.0	0.343	3.45	0 38	5.64

O: Organic Carbon; P: Phosphorus; N: Nitrogen; EC: Electrolytic conductivity

Analysis of heavy metals

Soil samples collected from different sites were analyzed for heavy metals. The highest values were obtained in samples from VAM Organics, Gajrola, samples and the least in KSTPS Korba samples (Table 2.2).

Table 2.2 Levels of heavy metals in soil samples

Site	*Metal (ppm)*							
	Cd	*Cr*	*Cu*	*Fe*	*Pb*	*Mn*	*Ni*	*Zn*
VAM organics,	24.9-38.4	36.7-89.3	2.0-121.1	8356.6-31873.0	312.4-444.8	168.4-516.4	93.7-128.3	45.7-268.4
Gajrola	T	T	T		T	N	T	T
DTPS	29.1-34.3	11.8-90.9	6.93-49.6	12470.8-26355.2	291.5-352.8	145.1-354.2	102.3-137.9	49.4-89.2
Dadri	T	T	N		T	N	T	T
ITPS	25.4-37.9	28.0-77.9	18.6-36.5	16229.9-24579.9	308.6-381.0	206.3-433.5	108.5-155.1	69.1-103.
	T	T	N		T	N	T	T
OIA	23.5-37.7	24.1-87.8	15.8-523.2	12876.7-27913.0	342.9-550.0	215.0-480.9	96.3-217.6	68.0-349.5
	T	T	T		T	N	T	T
KSTPS,	21.4-21.8	49.7-55.1	4.03-15.2	12269-16689	320.9-327.4	181.5-304.2	72.8-84.3	44.1-66.1
Korba	T	N	N		T	N	N	N

T: Toxicity possible, N: Normal

Raising of trap cultures for EM fungi

Pot (diameter 12 cm; height 10 cm) were used to establish the trap culture. The short roots and soil collected along with the EM from the various sites was mixed with 20% fly ash, 10% sand with respect to the volume of the soil sample. Plants used for trapping the fungi were maize, sorghum, tomato, chilli, and tree species of *Eucalyptus* and *Pinus roxburghii.*

Raising of trap cultures for AM fungi

Pot (diameter 11.3 cm; height 9.5 cm) were used to establish the trap culture. The roots collected from various sites were chopped and mixed with sterilized sand in the pots. Plants used for trapping the fungi were sorghum, tomato, leek, chilli, and onion.

The trap cultures raised were subjected to infectivity potential analysis (Gaur *et. al.*, 1998) after the first cycle (Table 2.3).

Table 2.3 Infectivity potential isolates after first cycle

Site	*Sample*	*Ip*/g substrate*
VAM Organics Gajrola	1	0.89
	2	0.23
	3	0.20
	4	0.9
	5	0.23
	6	0.47
DTPS, Dadri	1	0.8
	2	0.23
	3	0.25
	4	0.42
	5	0.13
	6	0.14
Indraprastha, TPS	1	0.6
	2	0.18
	3	0.22
	4	0.14
	5	0.17
Okhla Industrial Area	1	0.01
	2	0.137
	3	0.126
	4	0.12
	5	0.5
	6	0.106
	7	0.51
	8	0.22
	9	0.186
	10	0.11
	11	0.23

*I P= infectivity potential

Nancy - France

In France, ectomycorrhizal strains were collected during autumn 2000 from an ash dyke belonging to EDF, located near Pont à Mousson (54). The oldest parts of the dyke were abandoned 20 years ago and have been spontaneously colonized by a number of ectomycorrhizal (*Pinus, Populus, Alnus, Fagus*) and endomycorrhizal (*Salix, Alnus, Populus*) tree species (Table 2.4). Colonized ashes were sent to TERI for VAM strain isolation. Fruit bodies of ectomycorrhizal fungi were collected (Table 2.4). Five mycelial cultures were isolated from the fruitbodies. Their performance on ashes was further investigated.

Table 2.4 Trees and ectomycorrhizal fungi observed on Pont à Mousson ask dyke (France)

Trees	*Ectomycorrhizal fungi*
Populus sp.	*Suillus luteus* *
Tremula sp.	*Lactarius terminosus*
Betula sp.	*Lactarius sp.*
Alnus sp.	*Russula sp. 1*
Fagus sp.	*Russula sp. 2*
Salix sp.	*Russula sp. 3*
Pinus sp.	*Russula sp. 4*
Quercus sp.	*Paxillus involutus* *
Robinia pseudoacacia	*Hebeloma sp.*
	Leccinium sp. 1 *
	Leccinium sp. 2 *
	Leccinium scaber *

** indicates strains sucessfully isolated from fruitbodies*

To facilitate understanding of the behaviour of ectomycorrhizal fungi when grown in fly ash, their strains were selected from Nancy University's (Dr Michel Chalot) culture collection. Four strains were selected on basis of their resistance to heavy metals (Zn, Cu, Cd, Ni). Two are categorized as highly tolerant (*Paxillus involutus, Pisolithus tinctorius*), one as sensitive. These were considered bioindicators of ash toxicity. Three other strains, non-characterized in terms of heavy metal toxicity, have been selected because their physiology has been widely investigated.

Ectomycorrhizal strains isolated in France as well as heavy-metal tolerant strains (tested) from local collections (cf. first year report) were transferred to India, for evaluation on ashes in association with host plants (Task 3). The objectives were achieved; no more work was conducted in Task 1.

Collaboration

Joint visits were made to collect mycorrhiza from various sites in India and France. Investigators helped each other to characterize organisms based on specific expertise of both the groups. Logical selections of organisms were also done in consultation with each other.

3

Task 2 – Attempts to obtain EM strains better adaptable to ash pond

TERI – India

Selection of appropriate ectomycorrhizal strains adaptable to ash ponds

Experiment -1

Introduction

Species of ectomycorrhizal isolates are regular symbionts with several important tree species. Mass culturing of a fungal inoculum becomes the first and foremost requirement for plantation/nursery inoculations. In order to achieve good mass production, favorable physiological growth conditions for the fungus have to be standardized. The main objective of the present task was to determine the pH at which the maximum growth of the ectomycorrhizal isolate was obtained and also to study the range of pH tolerance of the culture.

Materials & Methods

Selection of isolates

Fungal isolates selected for the experiment were:

Bank Code	*Culture Name*	*Host*
EM-1145	*Amanita murina*	*Eucalyptus bridgesina*
EM-1185	*Hysterangium incarceratum*	*Eucalyptus sp.*
EM-1083	*Laccana fraterna*	*Eucalyptus globulus*
EM-1191	*Laccaria laccata*	*Eucalyptus sp.*
EM-1198	*Lactenous controversus*	*Populus euramericana*
EM-1197	*Lacterious controversus*	*Populus euramencana*
EM-1267	*Paxilus involutus*	*Eucalyptus darlympleana*
EM-1212	*Paxilus involutus*	*Populus euramericana*
EM-1081	*Pisolithus tinctorious*	*Eucalyptus tereticornis*
EM-1235	*Scleroderma flavidum*	*Eucalyptus camaldulensis*
EM-1233	*Scleroderma cepa*	*Eucalyptus sp*
EM-1290	*Pisolithus tinctonous*	*Eucalyptus tereticornis*
EM-1291	*Pisolithus tinctorious*	*Eucalyptus tereticornis*
EM-1292	*Scleroderma verucosum*	*Eucalyptus tereticornis, Silver oak*
EM-1283	*Scleroderma verucosum*	*Shorea roubasta*
EM-1293	*Pisolithus tinctorious*	*Eucalyptus tereticornis*

Plan of experiment

The cultures were regularly maintained on the modified Melin Norkan's medium (MMN) (Marx 1969) with agar. For the experiment to determine the optimum pH. The pH remained between 4.0 and 10.0. The pH was adjusted so as to be able to observe the optimum range and also the relative pH tolerance.

The MMN agar plates were inoculated with three equidistant mycelial agar discs cut from the edges of an actively- growing colony. The inoculated plates were incubated in a BOD incubator at 24 ° C till adjacent colonies touched each other. The growth of the colony was measured in terms of colony diameter using the software Image ProPlus® (Sundari and Adholeya 2001).

The pH of the substrate agar was recorded before inoculating the culture and after the completion of growth phase using the solid state pH meter (Orion 620).

Results & Discussions

The growth response of EM fungi in different pH regimes is represented by the graphs in Figures 3.1.1 – 3.1.16.

Any factor which affects the growth of the mycelium, will have a direct effect on the extent of colonization of new roots and thus needs to be clearly addressed, as it is a major function of the vegetative mycelium. pH is one factor which influences mycelial extension and growth. In our experiment all the isolates tested responded markedly to the substrate pH. All isolates except five showed a significant growth response at a single pH value. Colony characteristics remained unchanged all through the pH range. However one culture *Scleroderma verucosum* (EM-1283) was associated with a marked change in the colour of the medium presumably due to the secretion of phenolic compounds under stressed pH conditions. An essential finding of this experiment was that isolates can be classified into acidic, alkaline and intermediate. This may be used as a basis for selecting isolates for future applications.

Though *in vitro* studies indicate an effect of pH upon the growth of vegetative mycelium it must be kept in mind that, the *in vitro* response of the fungus may not always be the same when growing as a symbiont in association with the host.

Experiment - 2

Introduction

Ash ponds are commonly used to dispose coal ash in thermal power stations. To develop ectomycorrhizal (EM) isolates adaptable to ash ponds it is necessary to understand the impact of coal ash on them. The following experiment was aimed at understanding the impact of coal ash on EM fungi and also the ability of various isolates to take up heavy metals and elements as well.

Coal ash and its categories

Coal ash is a waste byproduct of coal-based thermal power plants generated as a result of combustion of pulverized coal. Coal ash may contain heavy metals such as As, Ba, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Zn. Coal ash is categorized into three classes with their properties described below:

Bottom ash

This ash is collected from the bottom of the furnace, after being ground in the clinker grinder unit. A relatively high carbon content and coarseness characterize it. About 20% of the total ash produced during power generation is of this type.

ESP ash

This refers to the ash collected from the hopper under the electrostatic precipitators (ESPs). This kind of ash has a relatively low carbon content and is very fine in texture. It comprises about 80% of the total ash produced.

Pond ash

Ash collected from the hoppers and the bottom of the furnaces is removed by a stream of water pumped through the ash through pipelines or slurry trenches. The ash slurry is deposited in a disposal area called an ash pond. After some time, the ash particles settle down due to gravity and water on the surface is siphoned off for reuse after treatment.

Materials and Methods

Selection of the isolate

Fungal isolates selected for the study, *Laccaria fraterna* EM-1083; *Pisolithus tinctorius* EM-1081; *Scleroderma cepa* EM-1233; *Pisolithus tinctorius* EM-

1290; *Scleroderma verucosum* EM-1283; *Pisolithus tinctorius* EM-1293 were obtained from the Centre for Mycorrhizal Culture Collection, Tata Energy Research Institute, New Delhi India, and were maintained in the modified Melin-Norkran (MMN) medium (Marx 1969).

Plan of experiment

Three types of ash were autoclaved dry (121° C, 1.2 kg/cm^2, 1 hour) twice at an interval of 24 hours and poured into deep-welled pertiplates (90 mm × 22 mm; diameter × height) @ 0%(w/v), 5%(w/v), 10%(w/v), 25%(w/v), 50% (w/v) , and 75% (w/v). MMN with 0.3% phytagel (Sigma Chemical Co. USA) as gelling agent was prepared separately, autoclaved and dispensed in fixed 60 ml volumes to each petriplate simultaneously.

Measurement of ash dependency

Three fungal discs of 7 mm diameter were cut from the edge of an actively-growing colony, placed equidistant in each of the petriplates and incubated in the dark at 24°C until the margin of the adjacent colonies touched each other. Fungal growth in terms of colony diameter was measured as a function of time (Sundari and Adholeya 2001) using the software Image Proplus®

Variance in the medium pH due to different levels of ash was recorded after autoclaving and at the time of harvesting the mycelia using a solid state pH meter (Orion 620).

Heavy metal analysis

After growth, the mycelium in each of the petriplates was harvested by washing with sodium citrate buffer (Doner and Becard 1991) at 37°C, and the mycelial dry weight of triplicate samples were determined after overnight air drying. Colonies were digested with 5 ml of HNO_3 and 1 ml of HF in a closed vessel at 170°C (Kalra 1989) using MARS5 (microwave accelerated reaction system 5), CEM Corp, for heavy metal analysis. The digested samples were analyzed for Al, Ni, Pb, Cr, Cd, Fe. The analysis was carried out by TJA Solutions AAS software version 1.14 (Unicam) Model SOLAAR M Series with GF 95 graphite furnace equipped with FS 95 auto sampler having Software SOLAAR M Data station version 8.12.

Results

Measurement of ash dependency

The growth response of isolates of the ectomycorrhizal fungi in the three types of coal ash at different amendment levels is represented by the graphs in Figures 3.2.1–3.2.6).

Laccaria fraterna (EM-1083)

This isolate has shown a typical growth pattern (Figure 3.2.1) in all the three types of ash. Optimum growth was observed at 10% ESP ash, 50% bottom ash, and 10% pond ash, respectively. The growth curve has clearly shown that very high ash concentrations of ESP ash and pond ash did not favour the growth of *Laccaria fraterna* (EM-1083) with respect to the control.

Pisolithus tinctorius (EM-1081)

This isolate has shown a typical growth (Figure 3.2.2) in two types of ashes. Optimum growth higher than the control was observed at 25% ESP ash, and 10% bottom ash. The growth remained unaffected by all the pond ash amendment levels, although the growth was restricted with respect to the control.

Pisolithus tinctorius (EM-1290)

Growth of the isolate (Figure 3.2.3) was enhanced in the bottom ash with respect to the control. Optimum growth was higher than the control was noted in pond ash and ESP ash at 50% amendment levels, respectively.

Scleroderma cepa (EM-1233)

This isolate has shown a typical growth (Figure 3.2.4) in ESP ash only. Optimum growth higher than the control was observed at 25% ESP ash. The growth remained unaffected by the pond ash amendments. Bottom ash acted as growth inhibitor with respect to the control.

Scleroderma verucosum (EM-1283)

Optimum growth of the isolate was observed (Figure 3.2.5) at 25% ESP ash, 10% bottom ash, and 25% pond ash respectively. The growth curve has shown that ESP ash has markedly enhanced the growth whereas both pond ash and bottom ash inhibited growth with respect to the control.

Pisolithus tinctorius (EM-1293)

No difference in growth (Figure 3.2.6) was noted due to change in bottom ash and ESP ash concentrations. However both of the ash types enhanced the growth with respect to the control. Pond ash showed a marked difference in growth due to a change in ash level. Growth has enhanced with an increase in ash concentrations.

Analysis of substrate pH

The variation in substrate pH due to ash amendments and subsequent growth of ectomycorrhizal isolates is represented in Table 3.1. The pH of the normal MMN medium with phytagel as gelling agent and with out any ash amendments was acidic. The pH of the medium remained around six at lower levels of ESP ash amendment, then decreased at higher levels of ash amendment. The pH alteration due to pond ash amendments was directly proportional to the level of ash amendment. Bottom ash amendment did not result in a significant change in medium pH at different levels.

Laccaria fraterna (EM-1083)

The pH of the medium was recorded at harvest. The final pH was highly acidic in the three types of ash amendment at almost all levels. Results indicated an inverse relationship between acidity and ash amendment level. The most acidic pH was recorded when there was no ash.

Pisolithus tinctorius (EM-1081)

At harvest pH was recorded highly acidic in all three types of ash amendment at almost all the levels. For all the three types of ashes maximum acidity was recorded at lower level of ash amendment.

Pisolithus tinctorius (EM-1290)

The most acidic pH was recorded at lower levels of ash amendment in ESP ash and bottom ash respectively at harvest. For pond ash, the most acidic pH was recorded at higher levels of ash amendment.

Pisolithus tinctorius (EM-1290)

In this case also the most acidic pH was recorded at lower amendment levels in all the three type of coal ash. The maximum reduction in pH was recorded when no ash was present.

Scleroderma verucosum (EM-1283)

The reduction in pH of the medium was more at lower levels of ash amendment. Subsequent reduction in substrate pH due to fungal growth was lower higher levels of ash amendments in all the three types of coal ash.

Scleroderma cepa (EM-1233)

In this case also reduction in pH of the medium was more at lower levels of ash amendment. Subsequent reduction in substrate pH due to fungal growth was lower in higher levels of ash amendment in all the three types of coal ash.

Heavy metal analysis

Aluminum, nickel, lead, chromium, cadmium, and iron within the fungal mycelia were analyzed using atomic absorption spectrometer.

Aluminum

In ESP ash and pond ash, aluminum uptake by *Laccaria fraterna* (EM-1083) was directly proportional to the ash amendment (Figure 3.3.1). Maximum aluminum uptake was noticed in 75% pond ash and least in 25% pond ash. In bottom ash, uptake was low at low ash concentrations and more or less similar at higher concentrations of ash. Fungal growth in ESP ash and pond ash was inversely proportional to aluminum uptake and optimum growth was recorded at 50% concentration of bottom ash.

Aluminum uptake in *Pisolithus tinctorius* (EM-1081) was directly proportional to pond ash concentration and inversely proportional to the fungal growth (Figure 3.4.1). There was no significant different in the uptake in the bottom ash amendment. In ESP ash, the maximum uptake was at the 75% amendment level where the fungal growth was least.

Aluminum uptake was linearly related with growth in *Pisolithus tinctorius* (EM-1290) and had a cone-shaped curve in pond ash and ESP ash amended media (Figure 3.5.1). In bottom ash, the fungal growth was directly proportional to the concentration of ash. In ESP ash, aluminum uptake was inversely related to growth.

Aluminum uptake by *Pisolithus tinctorius* (EM-1293) was high at intermediate levels and low at both lower and higher concentrations of bottom ash and ESP ash (Figure 3.6.1). In pond ash, aluminum uptake decreased with an increase in ash levels. Fungal growth decreased with at increase in the concentrations of bottom ash and ESP ash, whereas in pond ash, maximum growth was reported at 75% concentration with least aluminum uptake.

There was no significant uptake in aluminum by *Scleroderma cepa* (EM-1233) in any treatment except at the 75% bottom ash concentration level (Figure 3.7.1).

Aluminum uptake and subsequent growth of *Scleroderma verucosum* (EM-1283) decreased with increase in the concentration of ESP ash (Figure 3.8.1). Uptake was higher at both lower and higher concentrations of pond ash, and vice versa in bottom ash.

Nickel

Nickel uptake in *Laccaria fraterna* (EM-1083) was inversely proportional to pond ash and bottom ash and directly proportional to ESP ash concentrations (Figure 3.3.2). Maximum uptake was noticed in 25% pond ash and least in 25% ESP ash. In bottom ash and ESP ash maximum growth was found where nickel uptake was low and in pond ash uptake was directly proportional to growth.

Nickel uptake was high at the 50% level and comparatively low at both, 25% and 50% concentrations of pond ash and ESP ash and vice versa in the bottom ash amendments (Figure 3.4.2) in *Pisolithus tinctorius* (EM-1081). Maximum uptake was reported in 25% bottom ash where the fungal growth was highest and minimum uptake was reported in 75% pond ash where the fungal growth was the lowest.

Nickel uptake and growth of *Pisolithus tinctorius* (EM-1290) showed a direct relationship with pond ash (Figure 3.5.2) and ESP ash amended media. Growth increased linearly with ash concentrations with a subsequent high uptake in bottom ash amended media.

In *Pisolithus tinctorius (EM-1293)* nickel uptake and subsequent fungal growth decreased with an increase in bottom ash levels, and increased with an increase in pond ash levels (Figure 3.6.2). There was no significant difference in uptake either with respect to growth or ash concentrations in ESP ash amended media.

Nickel uptake by *Scleroderma cepa* (EM-1233) increased and subsequent growth decreased with an increase in bottom ash concentration levels (Figure 3.7.2). In ESP ash nickel uptake and subsequent fungal growth decreased with the increase in levels, and in pond ash, uptake decreased but growth did not changed with an increase in concentration.

In *Scleroderma verucosum* (EM-1283) nickel uptake increased and subsequent fungal growth decreased with increased levels of pond ash, and both metal uptake and fungal growth decreased with increased bottom ash amendments (Figure 3.8.2). Significant nickel uptake was noticed only in 50% concentration of ESP ash.

Lead

In *Laccaria fraterna* (EM-1083), higher lead uptake was found where growth was more in ESP ash and pond ash whereas lead uptake did not increase significantly with respect to growth in bottom ash amended media (Figure 3.3.3).

Lead uptake by *Pisolithus tinctorius* (EM-1081) was high in 50% but low in both 25% and 75% pond ash and ESP ash amended media (Figure 3.4.3). In bottom ash, the lead uptake and fungal growth showed a direct relationship. However, lead uptake had a negative impact on growth in pond ash amended media.

Uptake of lead and fungal growth in *Pisolithus tinctorius* (EM-1290) was directly related in pond ash and ESP ash amended media (Figure 3.5.3). In bottom ash, growth increased linearly and subsequent lead uptake was also high.

In *Pisolithus tinctorius (EM-1293)* lead uptake and subsequent fungal growth was high in 50% whereas it was low at the 25% and 75% level of pond ash (Figure 3.6.3). Uptake and subsequent growth decreased with an increase in ESP and bottom ash amendment levels.

No significant change in the uptake of lead by *Scleroderma cepa* (EM-1233) was noticed with a difference in levels of pond ash and bottom ash in the media (Figure 3.7.3), although uptake was more in bottom ash in comparison to pond ash. Uptake and the subsequent fungal growth decreased with an increase in the level of ESP ash.

In *Scleroderma verucosum* (EM-1283), uptake of lead decreased with increased ash amendment levels, but the uptake at the highest level of ash amendment both in pond ash and bottom ash was more (Figure 3.8.3). Uptake and subsequent fungal growth decreased with increasing levels of ESP ash.

Chromium

In *Laccaria fraterna* (EM-1083), uptake of chromium was directly proportional to ash amendments in ESP ash and pond ash, and inversely proportional to bottom ash amendment levels (Figure 3.3.4). Growth was related inversely to chromium uptake in case of ESP ash and pond ash amendments. In bottom ash, the pattern was nonlinear however increased growth was observed where chromium uptake was low.

There was no significant difference in uptake of chromium by *Pisolithus tinctorius (EM-1081)* at different ESP amendment levels (Figure 3.4.4). In bottom ash and pond ash, chromium uptake was high at the 50% level, and low at both 25% and 75% levels of ash amendments.

In *Pisolithus tinctorius* (EM-1290), growth followed a cone-shaped curve as shown in the graph (Figure 3.5.4) with respect to pond ash and ESP ash levels. In ESP ash, uptake decreased with increase in concentration, and uptake was linearly related to growth in pond ash amended media. In bottom ash, growth increased linearly with chromium uptake.

Chromium uptake by *Pisolithus tinctorius* (EM-1293) showed a similar pattern with respect to pond ash and ESP ash amendment levels as shown in the graph (Figure 3.6.4). Uptake increased with an increase in the bottom ash amendment levels with a subsequent decrease in fungal growth.

In *Scleroderma cepa* (EM-1233), uptake of chromium increased but subsequent fungal growth remained unchanged with increase in bottom ash amendment levels, and vice versa with pond ash amendment levels (Figure 3.7.4). There was no significant difference in uptake with respect to ash concentrations in ESP ash amended media.

Uptake of chromium in *Scleroderma verucosum* (EM-1283) increased with increase in the ESP ash and bottom ash concentration but decreased at the 75% level where the subsequent fungal growth was also low (Figure 3.8.4). In pond ash, uptake decreased with increased concentration but at the 75% level of pond ash amendment there was increased uptake with a decrease in growth.

Cadmium

The cadmium uptake and growth in *Laccaria fraterna* (EM-1083) followed a cone-shaped curve with respect to concentrations of bottom ash (Figure 3.3.5). Growth and cadmium uptake were inversely related with respect to concentration of pond ash.

In bottom ash, the cadmium uptake was inversely related to the ash amendment levels in *Pisolithus tinctorius* (EM-1081) (Figure 3.4.5). In pond ash, uptake was high in the 50%, but lower in both 25% and 75% levels of pond ash. Cadmium uptake was found linearly related to the fungal growth in bottom ash amended media.

Uptake of cadmium in *Pisolithus tinctorius* (EM-1290) decreased with an increase in the concentration of ashes in all the three ash types (Figure 3.5.5). Growth followed a cone-shaped curve with respect to the concentrations of pond ash and ESP ash and increased linearly with respect to the concentrations of bottom ash.

Cadmium uptake in *Pisolithus tinctorius* (EM-1293) increased and the subsequent fungal growth remained unchanged with an increase in the concentration of ESP ash and vice versa in pond ash (Figure 3.6.5). However, cadmium uptake was higher both at the 25% and 75% levels of bottom ash.

Cadmium uptake in *Scleroderma cepa* (EM-1233) increased with an increase in ESP ash and bottom ash levels (Figure 3.7.5) and growth decreased with an increase in ESP ash but remained unchanged with an increase in bottom ash. Uptake was both higher at both, the 25% and 75% levels of pond ash.

In *Scleroderma verucosum* (EM-1283), cadmium uptake and subsequent growth decreased with an increase in the concentrations of pond ash (Figure 3.8.5). Cadmium uptake increased with an increase in the levels of bottom ash. Uptake and subsequent fungal growth was high at the 25% level of ESP ash and low at the 75% concentration of ESP ash.

Iron

Iron uptake in *Laccaria fraterna* (EM-1083) was found to be inversely related to growth in bottom ash, ESP ash and pond ash amendment levels (Figure 3.3.6).

Both iron uptake and growth of *Pisolithus tinctorius* (EM-1081) were linearly related and inversely proportional to the concentrations of bottom ash (Figure 3.4.6). Uptake showed a trend similar to that in bottom ash and ESP ash, however it was significantly high in ESP ash amended media. Uptake was insignificant with respect to the levels of pond ash.

Uptake of iron and subsequent mycelial growth in *Pisolithus tinctorius* (EM-1290) followed a direct relationship and a cone-shaped curve was obtained with respect to levels of pond ash (Figure 3.5.6). In ESP ash, uptake decreased with an increase in concentration. Maximum uptake was observed at the level in 75% bottom ash amended media where the subsequent fungal growth was also highest.

In *Pisolithus tinctorius* (EM-1293), iron uptake increased and the subsequent fungal growth decreased with an increase in the concentration of bottom ash (Figure 3.6.6). No significant different in uptake either with respect to growth or ash concentration was observed in pond ash amended media.

Iron uptake in *Scleroderma cepa* (EM-1233) decreased and subsequent fungal growth increased with an increase in the levels of pond ash (Figure 3.7.6). Uptake and subsequent growth decreased at the 25% and 50% level of ESP ash, but uptake was more at the 75% level where the subsequent mycelial growth was less. Uptake was less both at both the 25% and 75% levels of pond ash.

Iron uptake in *Scleroderma verucosum* (EM-1283) and subsequent growth decreased with an increase in the levels of pond ash. Uptake increased and growth decreased with increases in the levels of ESP ash and bottom ash (Figure 3.8.6). Iron uptake was however very low insignificant in maximum concentration of three types of ashes where the subsequent growth was also very less.

Nancy - France

(1st year)
A screening experiment was conducted using seven ectomycorrhizal strains to estimate their survivability upon ash substrate (Indian ashes from Korba) assumed to be toxic. The results already recorded with four isolates showed that the presence of ashes does not hinder the growth of any strain. Instead hyphal growth was strongly stimulated by ashes incorporated in the liquid nutrient medium at all concentrations tested (from 10% to 50%) (Fig. 1a-d). The growth of the isolate characterized as 'heavy metals sensitive' was not obviously inhibited either (experiment in progress). This initial result threw light on two interesting aspects. Firstly, ashes are probably not toxic to the growth and development of most mycorrhizal strains suggesting that mycorrhizal fungi can be successfully inoculated and benefit plants. Secondly, some strains tested are showing acidification of the substrate, indicating a possible alteration of the ashes by hyphae via proton exchange.

To estimate the toxicity of ashes, biotest data were completed by extensive mineral analysis of ash samples from the Korba power station. Ashes are discharged both by wet discharge and dry discharge methods; both samples were analyzed separately (Annexure 1, 2). On close observation and a comparison between the ashes of the wet discharge and dry discharge methods, it is revealed that, in the ashes the concentrations of heavy metal ions, though high, do not surpass the limits of toxicity set for soils. Except in the case of the two metals, nickel and chromium, whose concentration is close to the limits no other metals ion (where comparable data is available from literature) is anywhere near toxic limits. After discussion with experts working in the area of metal toxic sites, it was concluded that, given the pH of the ashes, only one metal ion, cadmium, could possibly be suspected for higher availability to plants than, as suggested by the chemical extraction procedures from standard protocols used for soil analysis. By contrast, though the concentrations of Ni and Cr seem to be near the limits, these metals ions should not be toxic given their bioavailability. Therefore cadmium bioavailability in ashes will be assessed (using radiolabelled ions) during the coming months to predict if there is any risk, even slight of excess available cadmium in ashes.

Both approaches, biological and analytical, suggest that ashes are compatible to the introduction of plants and mycorrhizal fungi. This preliminary conclusion needs to be confirmed.

(2nd year)

Toxicity of ashes

Bioavailability of critical elements

It was concluded, in the earlier report, that, given the pH of the Korba ashes (India), only one metal ion, cadmium, could possibly be suspected as being available to plants than suggested by the chemical extraction procedures from standard protocols used for soil analysis. Therefore cadmium bioavailability in ashes has been assessed to identify even a slight risk of excess available cadmium associated with ashes. Since the Ni concentration in ashes was near the recommended limits in agricultural soils, its bio-availability was assessed too. This work was conducted in collaboration with Guillaume Echevarria (ENSAIA, Nancy).

The ability of cadmium and nickel from ashes to enter the food chain as a result of a root-ash interaction was assessed using the Ion Exchange Kinetic method (IEK). An isotope of the element under investigation is introduced to the ashes and isotopic exchange of the element is studied at fixed time intervals. Results are extrapolated to asses the mobility and hence the availability of this element. The data for nickel and cadmium are presented in Table 3.2 (a and b respectively). All parameters recorded suggest that Ni and Cd bio-availability is either low or very low.

Table 3.2 Bioavalabiliy of nickel (a) and cadmium (b) from ashes assessed using the Ion Exchange Kinetic method (IEK). An isotope of the element under investigation is introduced in the ashes and isotopic exchange of the element is studied at fixed time intervals.

A)

Factor	*Definition*	*Value*	*Interpretation*
Cni	Intensity factor: Instantaneously available soil Ni	0.001-0.0023 mg/l	Low transfer
E_t	Quantity factor: Amount of element isotopically exchanged	0.066 mg/kg	<1 is low
Ni_L	Capacity factor: Buffering capacity	70 l/kg	Good
Ni total	Total element composition	50.8 mg/kg	Little high
N	Extrapolation	0.34	Fairly stable

B)

Factor	*Definition*	*Value*	*Interpretation*
CCd	Intensity factor: Instantaneously available soil Ni	0.0001mg/l	Very low - detection limit
E_t	Quantity factor: Amount of element isotopically exchanged	0.001mg/kg	Very low
Cd_L	Capacity factor: Buffering capacity	82 1/kg	Good
Cd total	Total element composition	0.26mg/kg	Low
N	Extrapolation	0.155	Fairly stable not high

Microscopic characterization of ashes and distribution of major cations

Since none of the chemical elements quantified in bulk ashes appeared to be present at a concentration suspected to be toxic, the distribution of these ions was investigated with a microprobe attached to an electron microscope to assess the ashes heterogeneity.

The Korba ashes are mainly constituted of irregular material (Fig 1a) among which spherical elements can be frequently identified (Fig 1b). The distribution of ions (Al, Si, Fe, K, Ca, S, Na, Ti) has been recorded in numerous samples. Al and Si being major elements are distributed evenly throughout the ashes irrespective of its constituents (irregular material or spherical elements) (Fig 1c, d). The same conclusion was reached about the minor elements analyzed, each of them appeared to be evenly distributed throughout the ashes; no accumulation sites could be evidenced. The only noticeable exception was Fe. Indeed, while Fe as other cations was detected all over the ash particles, some spherical elements accumulating higher concentrations of Fe could be detected (Fig 1e). After magnification, their surface appeared covered with typical iron ornament (Fig 1f). While such ornamented elements are noticeable they are rare and most probably insignificant in regard of plant and/or fungus nutrition.

Preliminary conclusion

According to the original planning, task 2 would not extend into the third year. From the data collected during years 1 and 2 we were not able to identify a major heavy metal toxicity problem related to Korba ashes. The following points emerged:

- The presence of ashes was not hindering the growth of strains tested including isolates characterized as 'heavy metals sensitive'.
- In the ashes the concentrations of heavy metal ions, though high, did not exceed the limits of toxicity set for soils.
- The cations are evenly distributed throughout the ashes. The ash composition seems to be very homogeneous.
- All parameters recorded suggest that Ni and Cd bioavailability is low or very low.

Such preliminary conclusions need to be confirmed by short term and long term field experiments (i.e. aluminium toxicity is highly dependent on pH and could be difficult to predict on long term).

(3rd year)
According to the original planning task 2 was not extended to the third year. A joint paper is being prepared presenting these data.

Collaboration

Results obtained were discussed during exchange visits and arrived on guidelines for future investigations.

Figure 1a

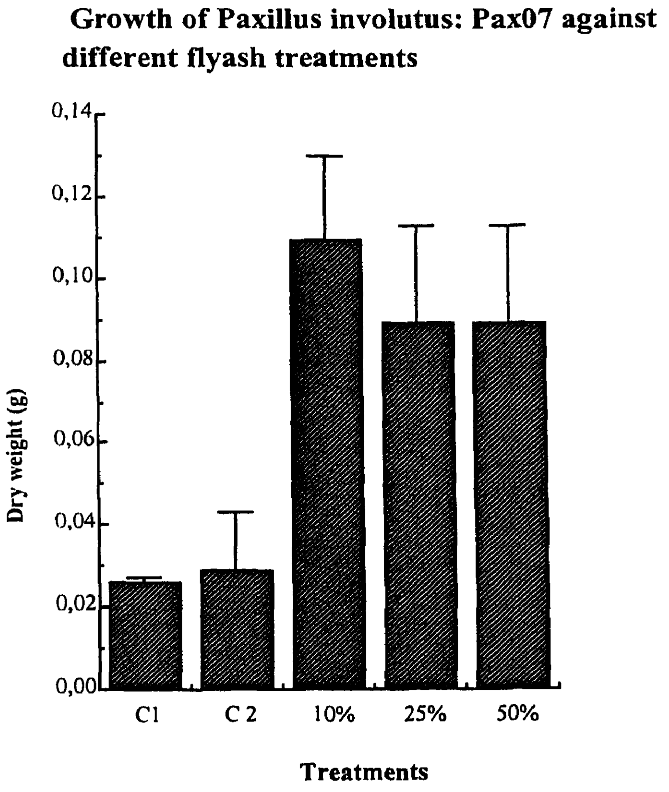


Figure 1b

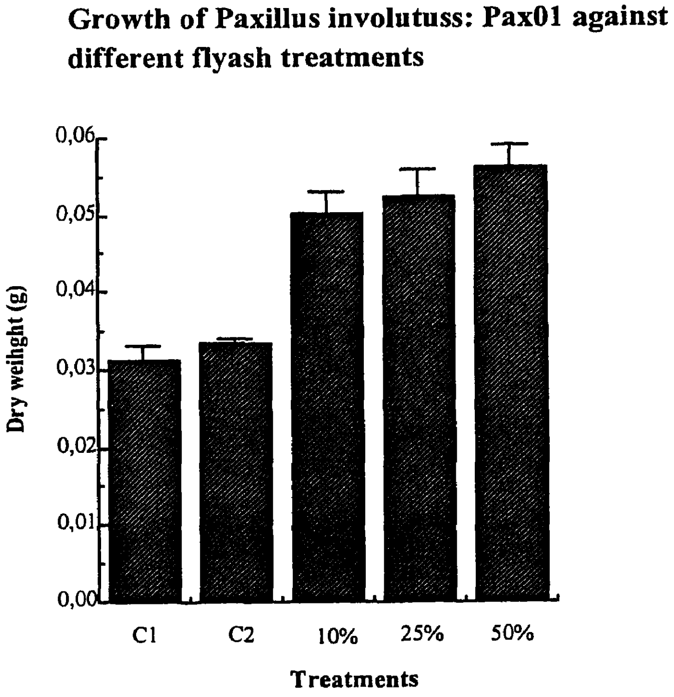


Figure 1c

Growth of Paxillus involutus: picou against different flyash treatments

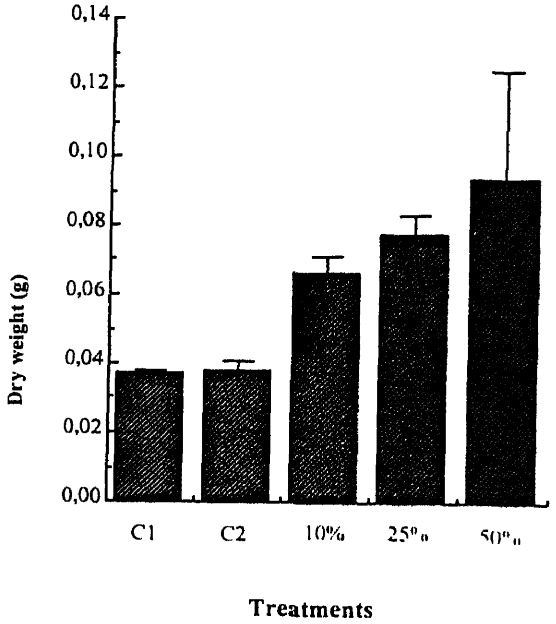


Figure 1d

Growth of Pisolithus tinctorius: Pt 2144 against different flyash treatments

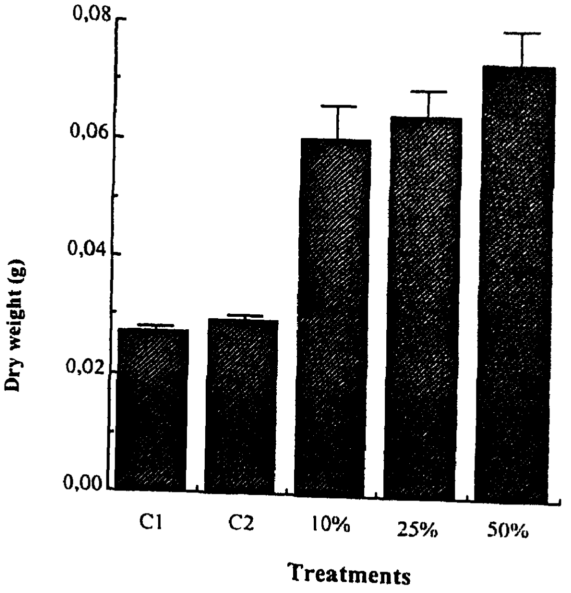
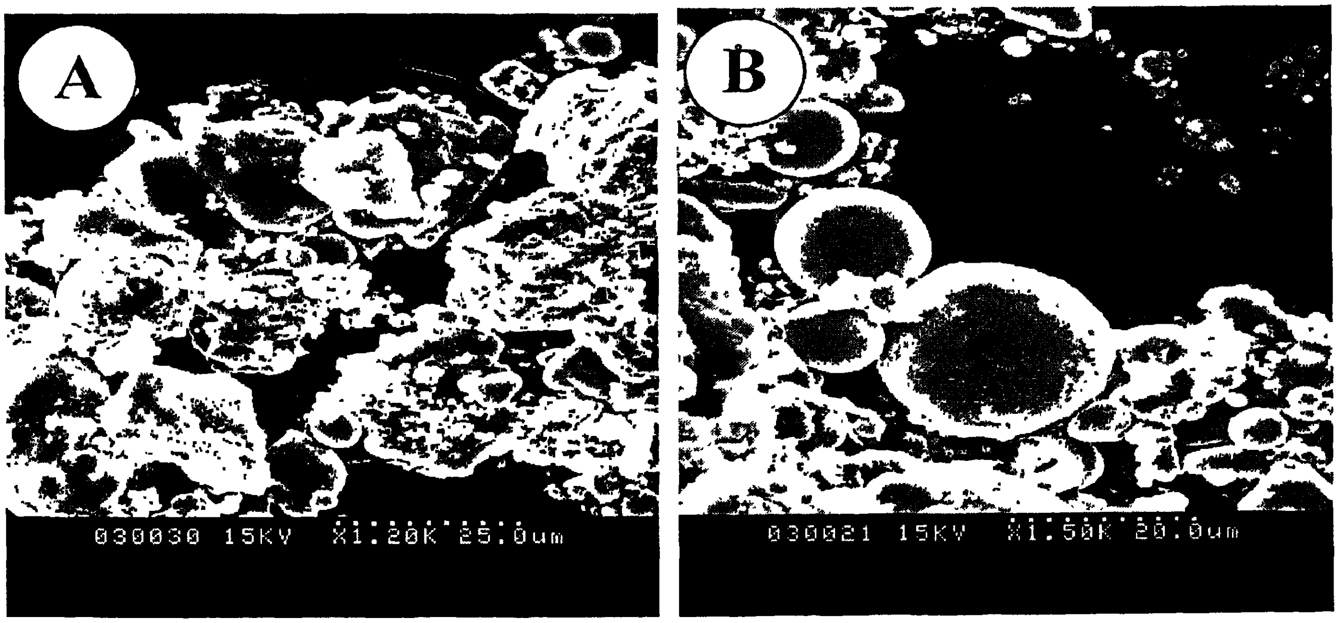


Figure 1: Microscopic observations of Korba ashes. Irregular material (A) among which spherical elements can be frequently identified (B) Al (C) and Si (D) are distributed evenly throughout the ashes. Some spherical elements accumulating higher concentrations of Fe could be detected (E), after magnification, their surface appeared covered with typical iron ornaments (F).



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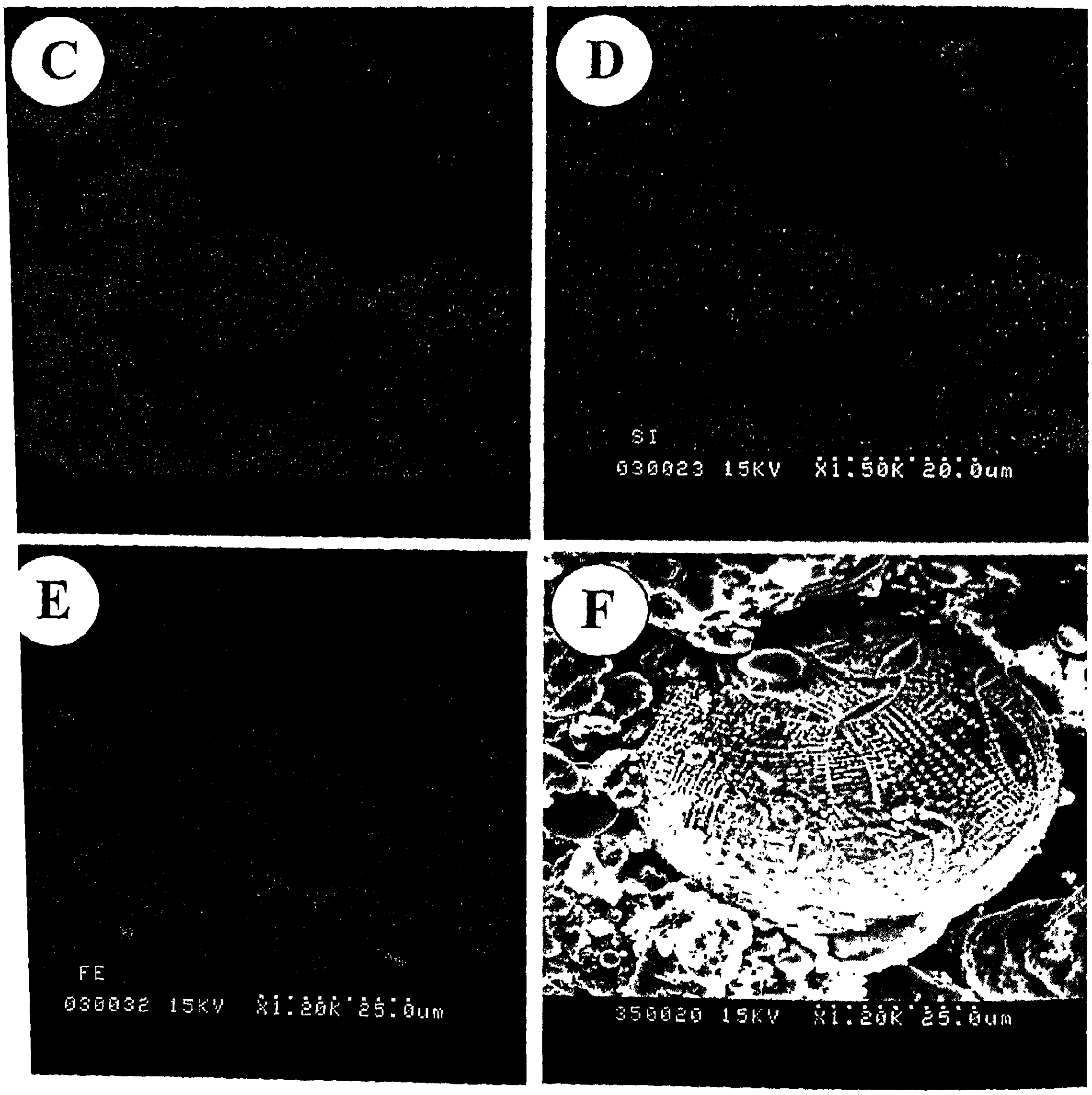
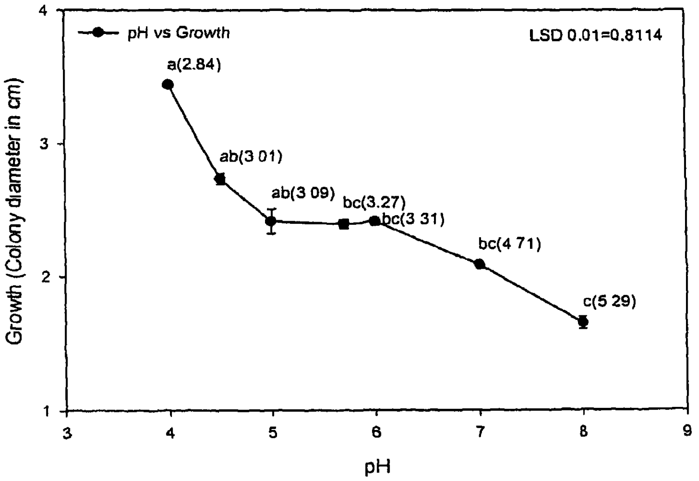
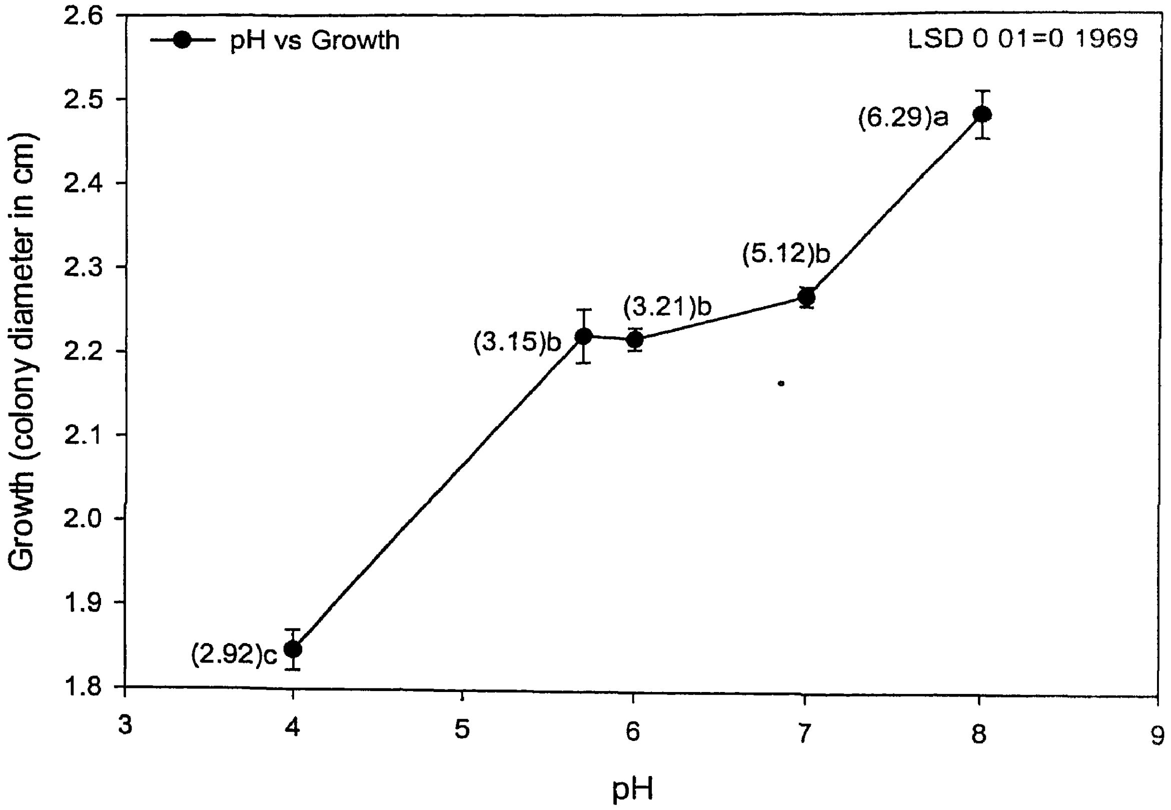

C
D
SI
030023 15KV X1.50K 20.0um
E
FE
030032 15KV X1.20K 25.0um
F
350020 15KV X1.20K 25.0um

Figure 3.1.1Growth of *Amanita murina* (EM-1145) in different pH regimes



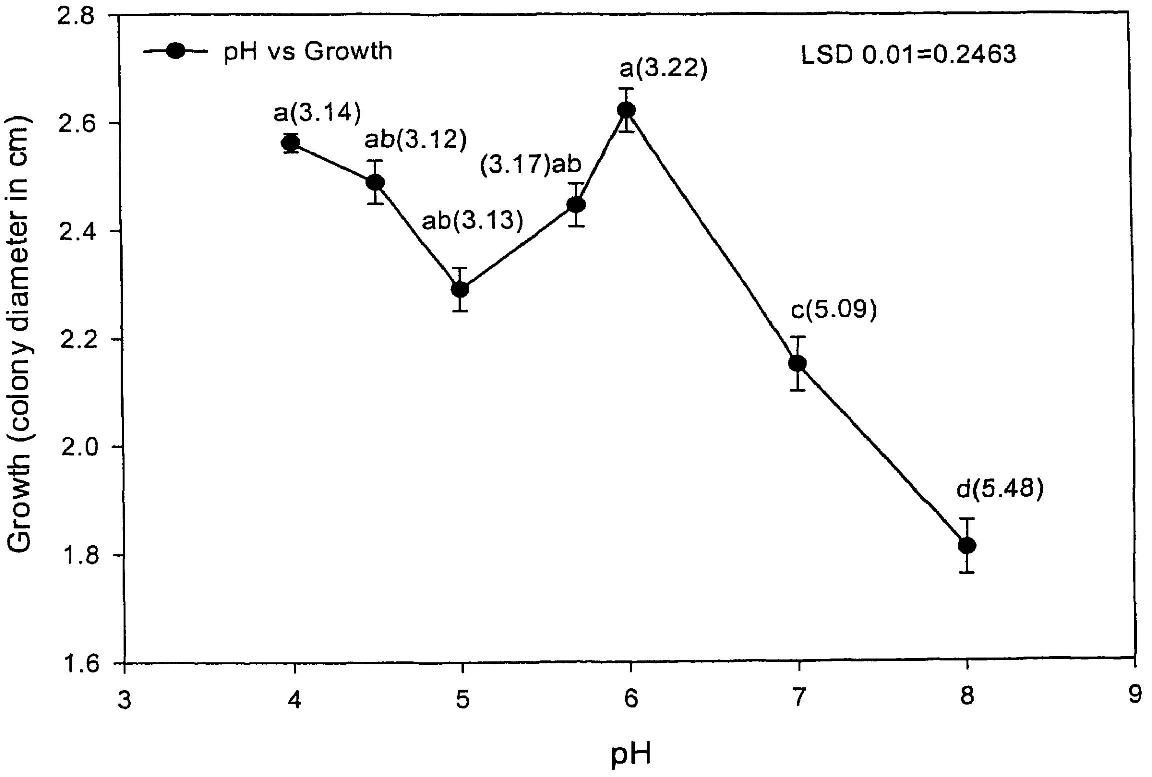
The value written in the parentheses at each point denotes the changed substrate pH.

Figure 3.1.2 Growth of *Hysterangium incarceratum* (EM-1185) in different pH regimes



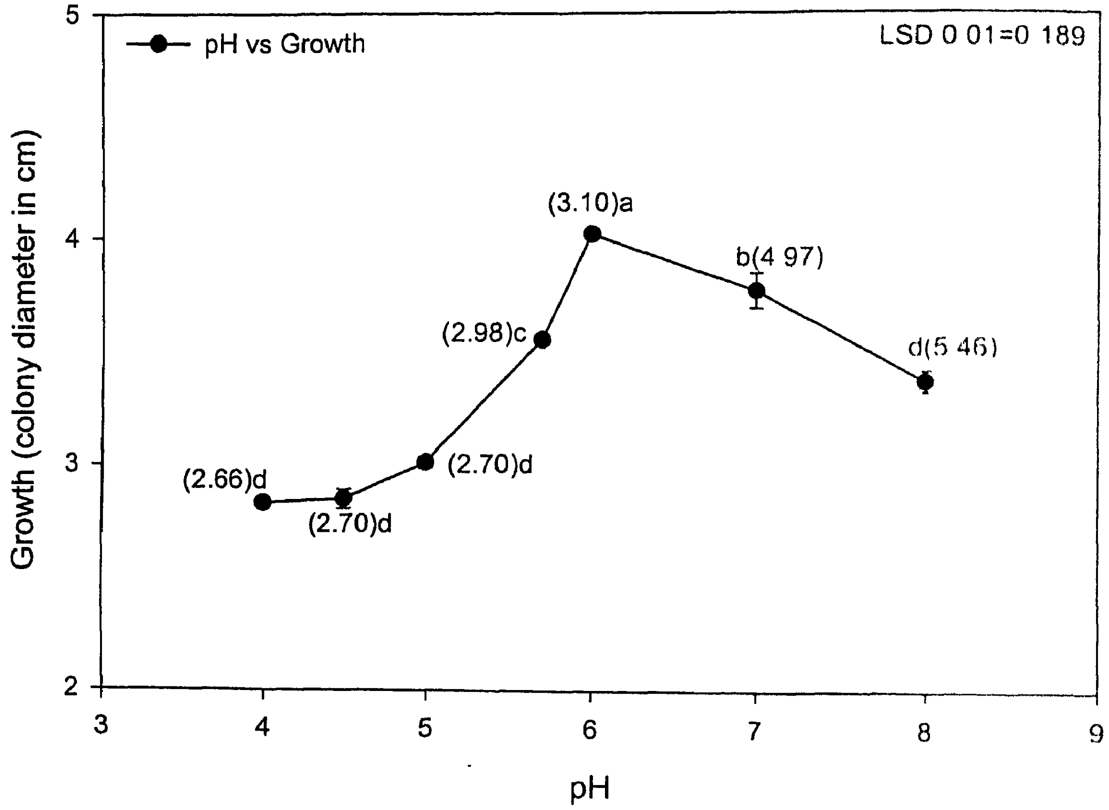
The value written in the parentheses at each point denotes the changed substrate pH.

Figure 3.1.3 Growth of *Laccaria fraterna* (EM-1083) in different pH regimes



The value written in the parentheses at each point denotes the changed substrate pH.

Figure 3.1.4 Growth response of *Laccaria laccata* (EM-1191) in different pH regimes



The value written in the parentheses at each point denotes the changed substrate pH.

Figure 3.1.5 Growth of *Lacterius controversus* (EM 1198) at different pH regimes

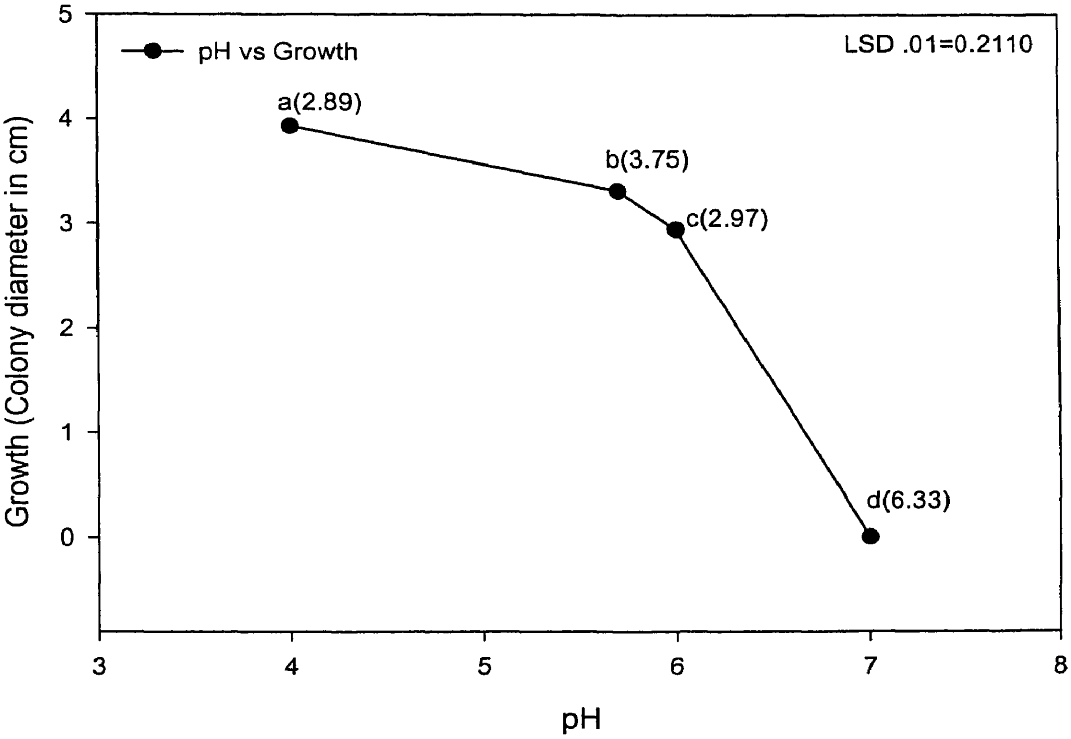
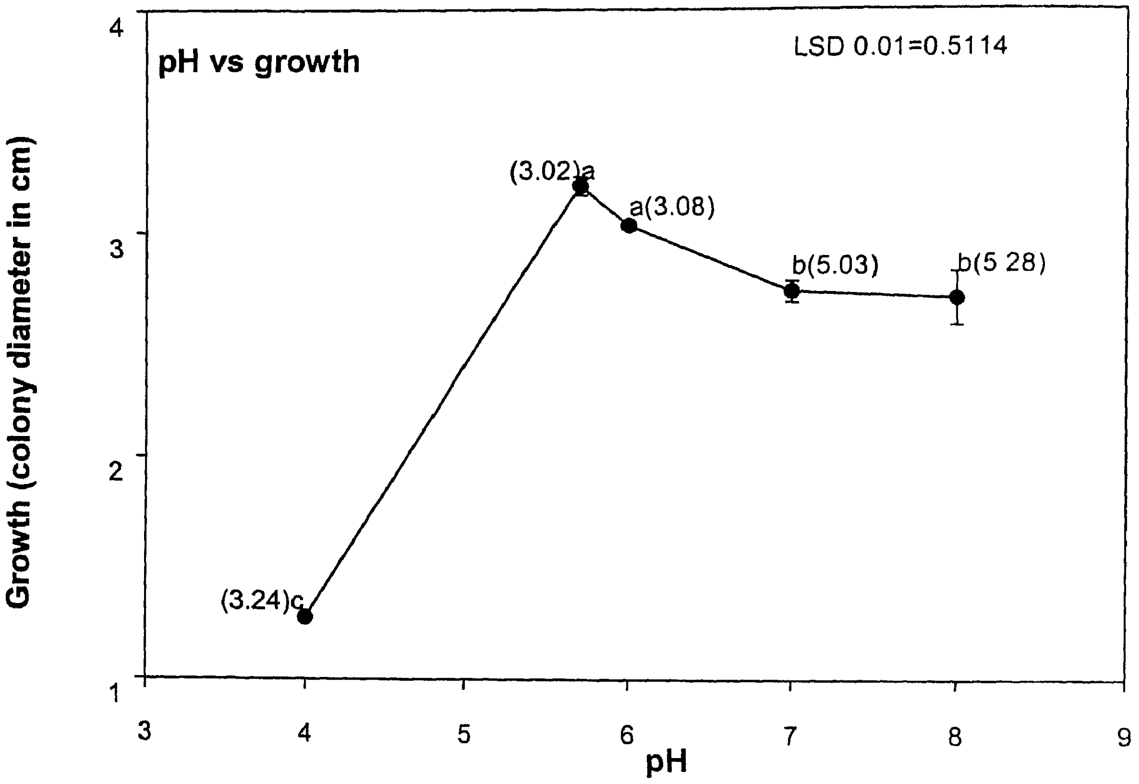
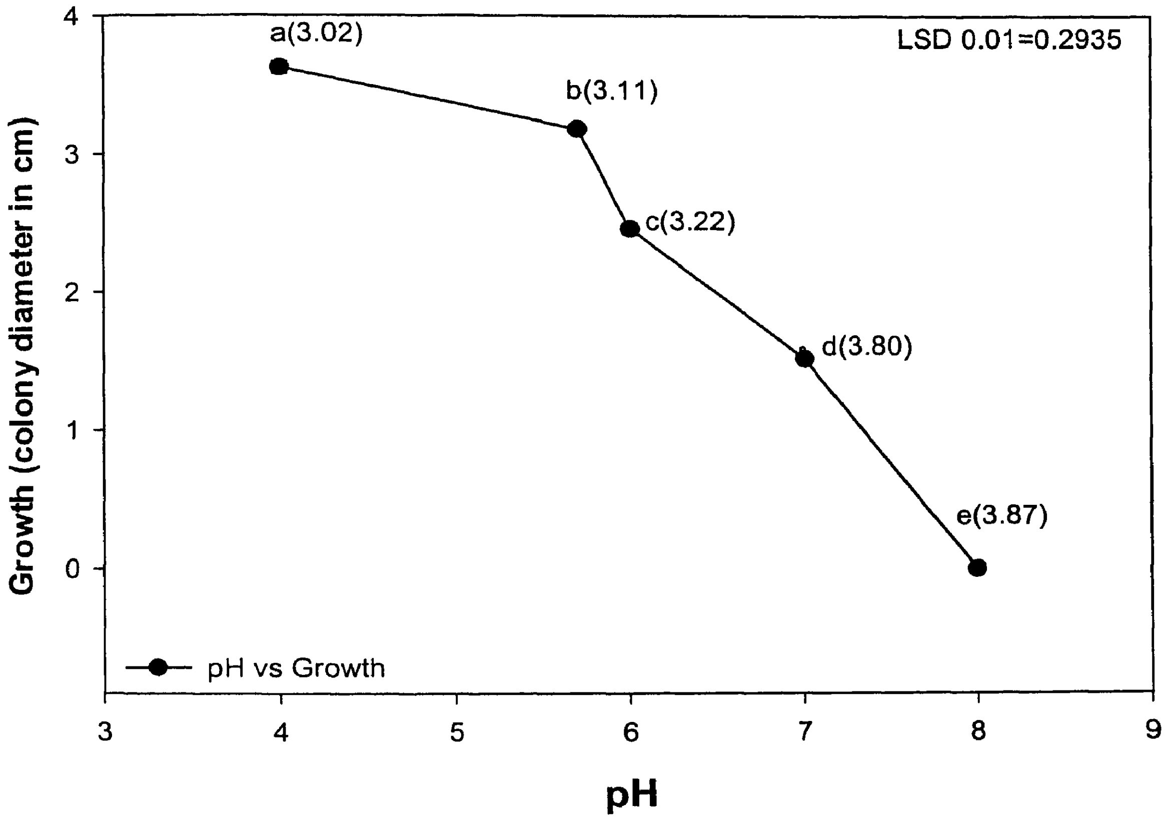


Figure 3.1.6 Growth curve of *Lacterius controversus* (EM-1197) in different pH regimes



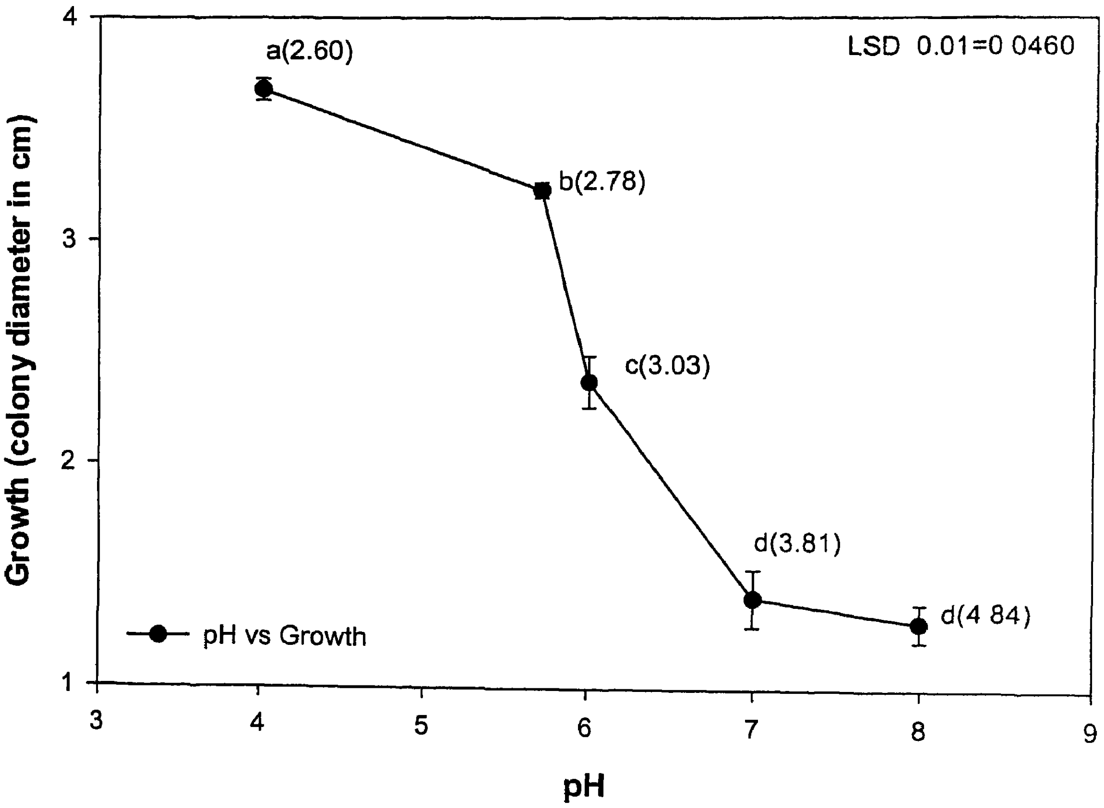
The value written in the parentheses at each point denotes the changed substrate pH

Figure 3.1.7 Growth of *Paxillus involutus* (EM-1267) in different pH regimes



The value written in the parentheses at each point denotes the changed substrate pH.

Figure 3.1.8 Growth of *Paxillus involutus* (EM-1212) in different pH regimes



The value written in the parentheses at each point denotes the changed substrate pH.

Figure 3.1.9 Growth of *Pisolithus tinctorius* (EM-1081) in different pH regimes

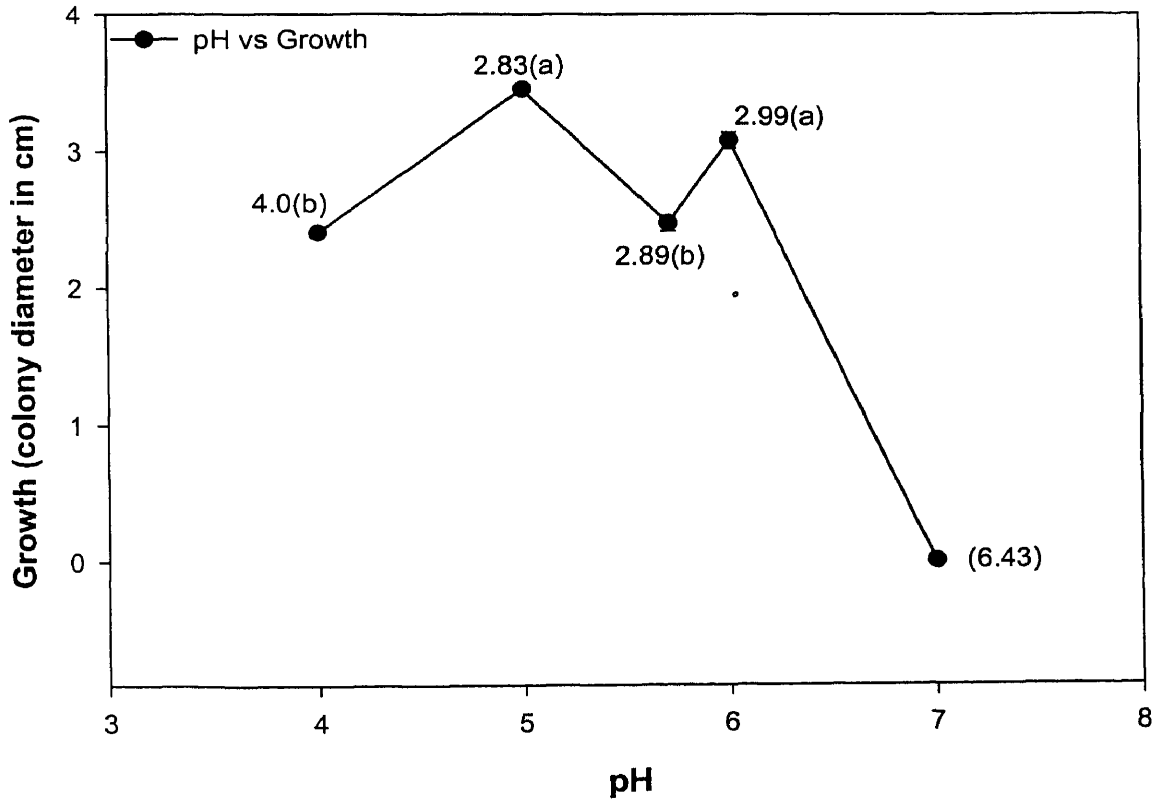


Figure 3.1.10 Growth of *Scleroderma flavidum* (EM-1235) in differe

pH regimes

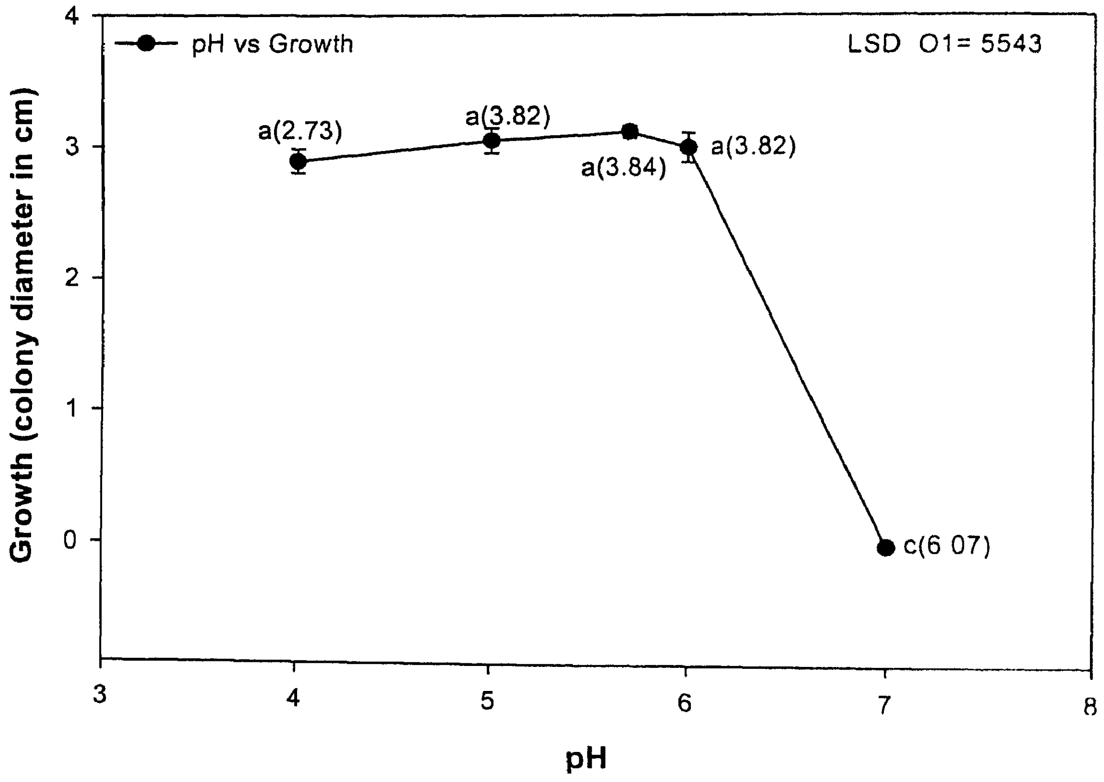
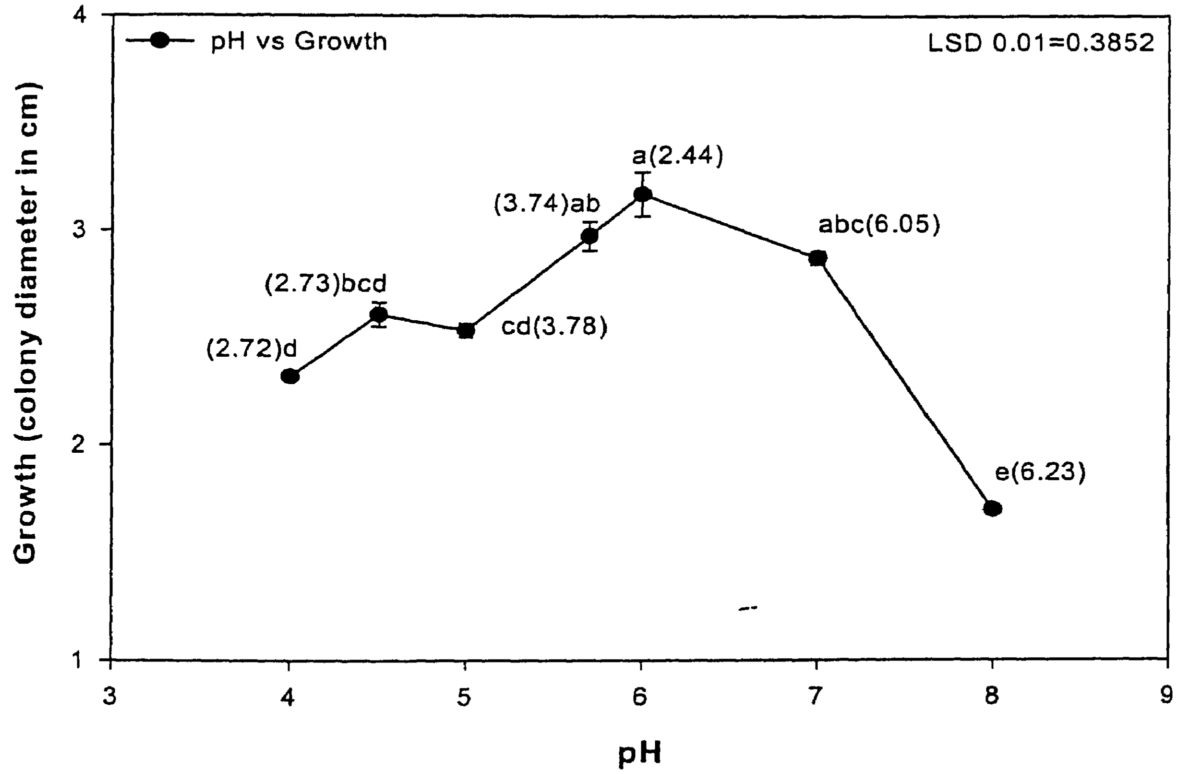
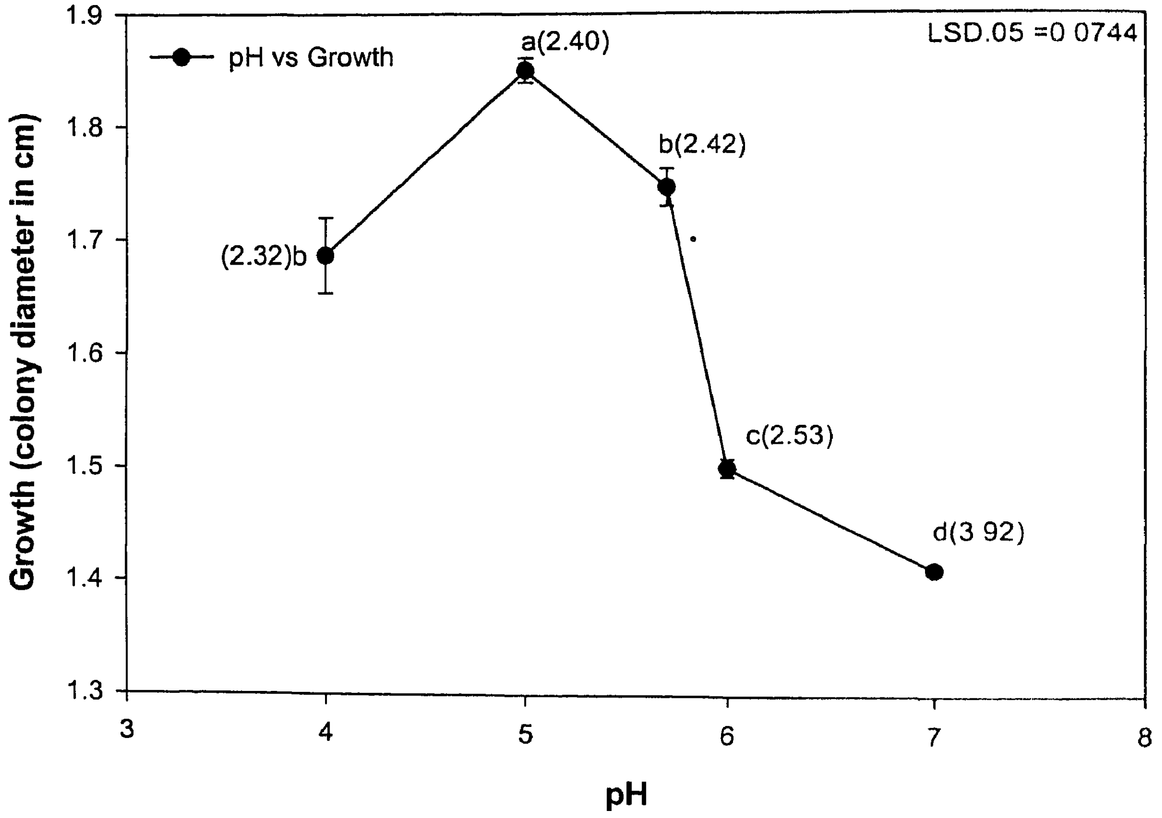


Figure 3.1.11 Growth of *Scleroderma cepa* (EM-1233) in different pH regimes



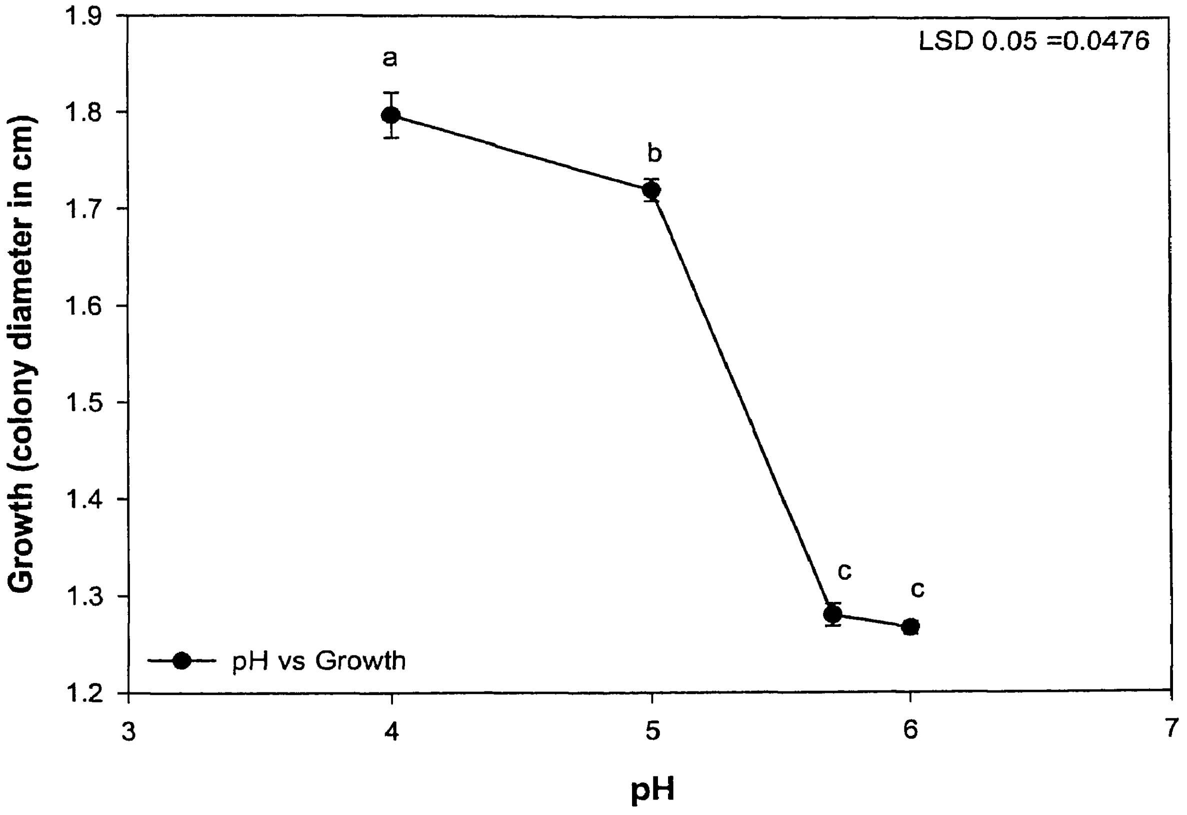
The value written on the parentheses at each point shows changed substrate pH.

Figure3.1.12 Growth of *Pisolithus tinctorius* (EM-1290) in different pH regimes



The value written in the parentheses at each point shows changed substrate pH

Figure 3.1.13 Growth of *Pisolithus tinctorius* (EM-1291) in different pH regimes



The value written in the parentheses at point shows changed substrate pH.

Figures 3.1.14 Growth of *Scleroderma verucosum* (EM-1292) in different pH regimes

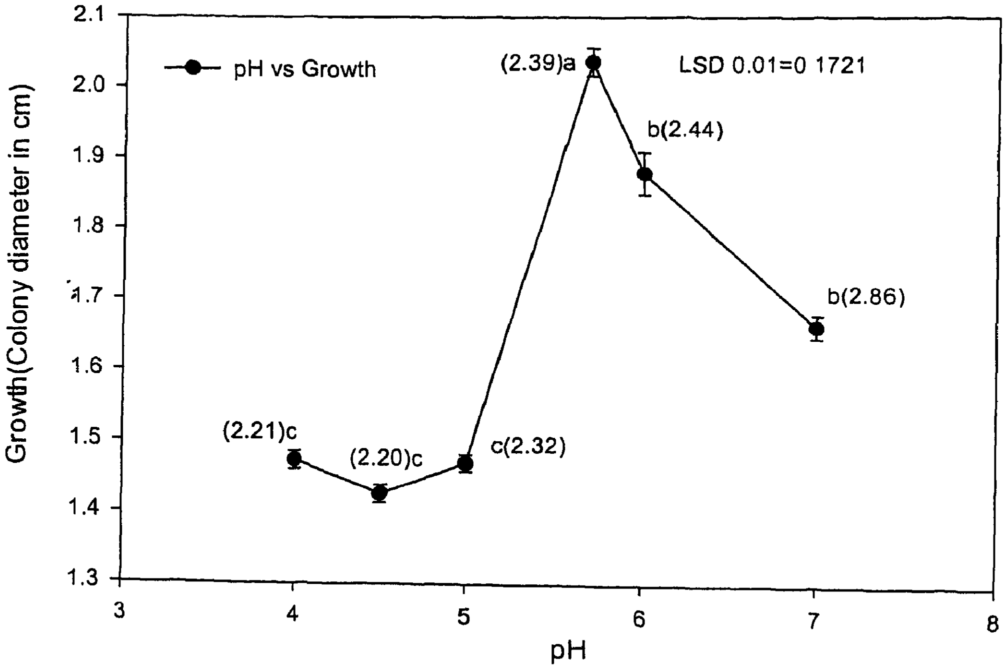
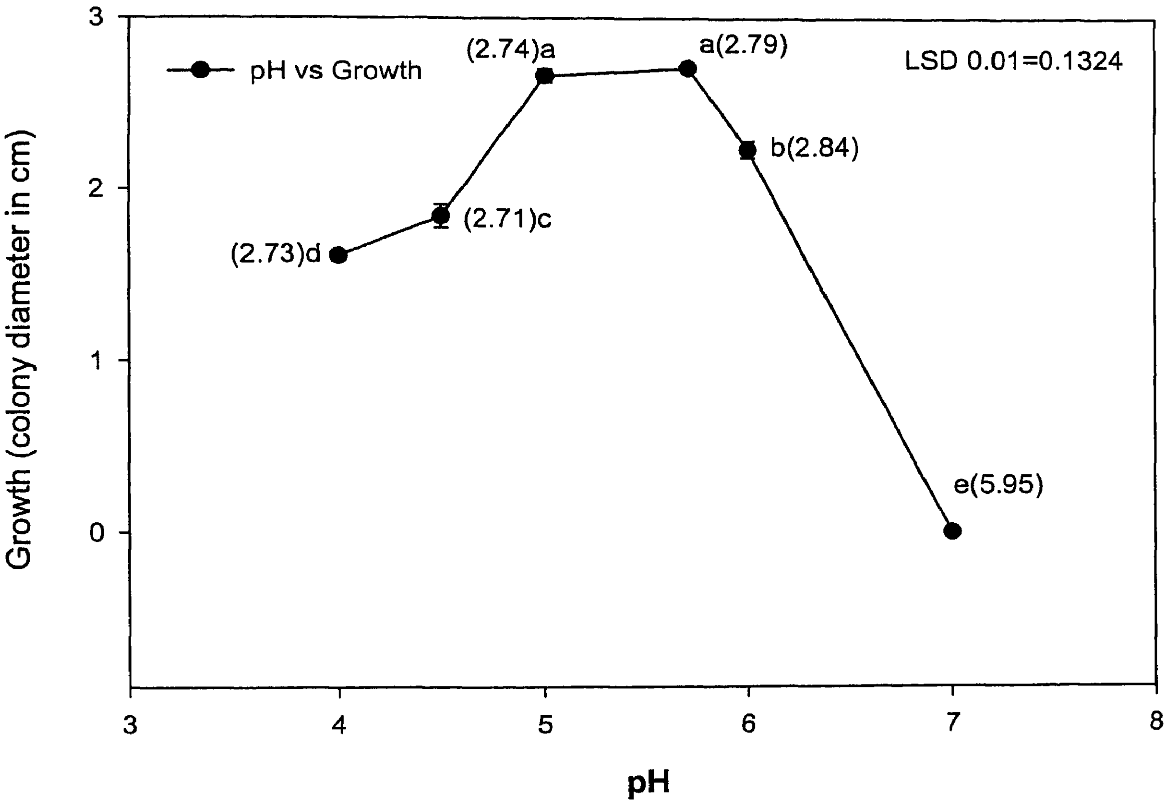
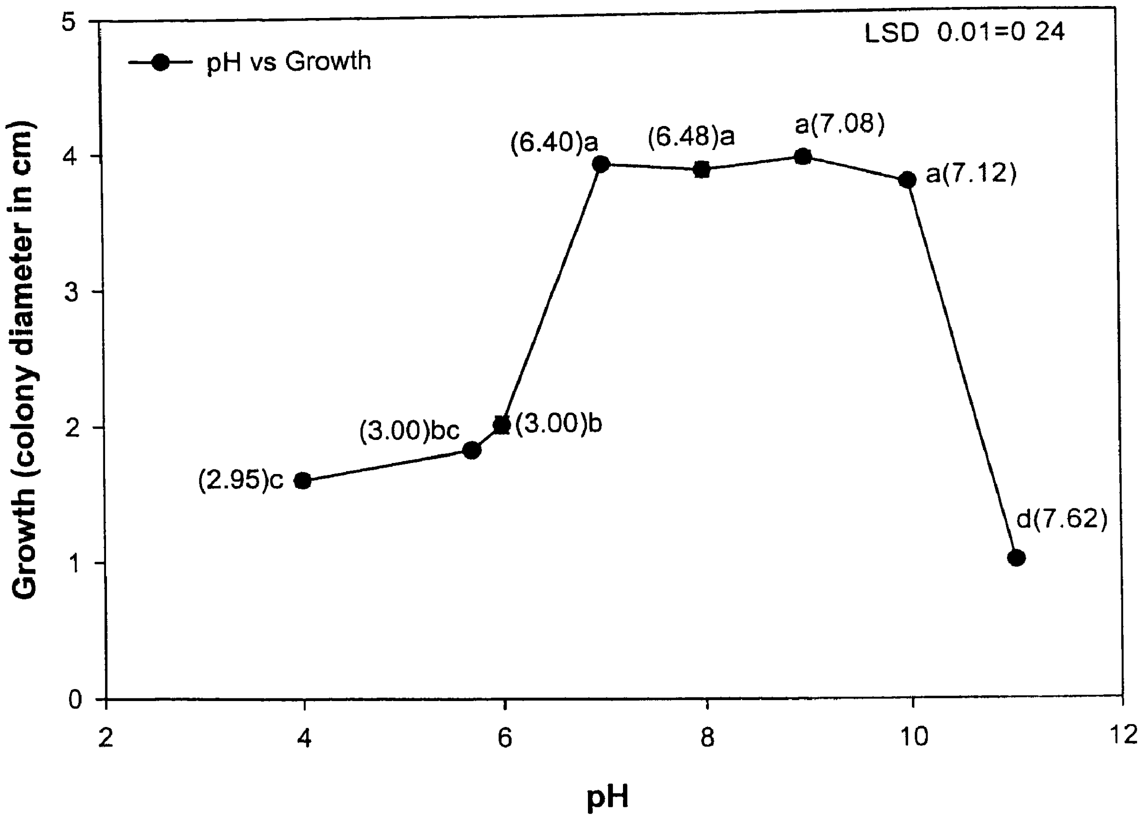


Figure3.1.15 Growth of *Scleroderma verucosum* (EM-1283) in different pH regimes



The value written in the parentheses at each point shows changed substrate pH.

Figure 3.1.16 Growth of Pisolithus tinctorius (EM-1293) in different pH regimes



The value written in the parentheses at each point shows changed substrate pH

Figure 3.2.1 Growth response of *Laccaria fraterna* (EM-1083) at different concentrations of three types of ashes

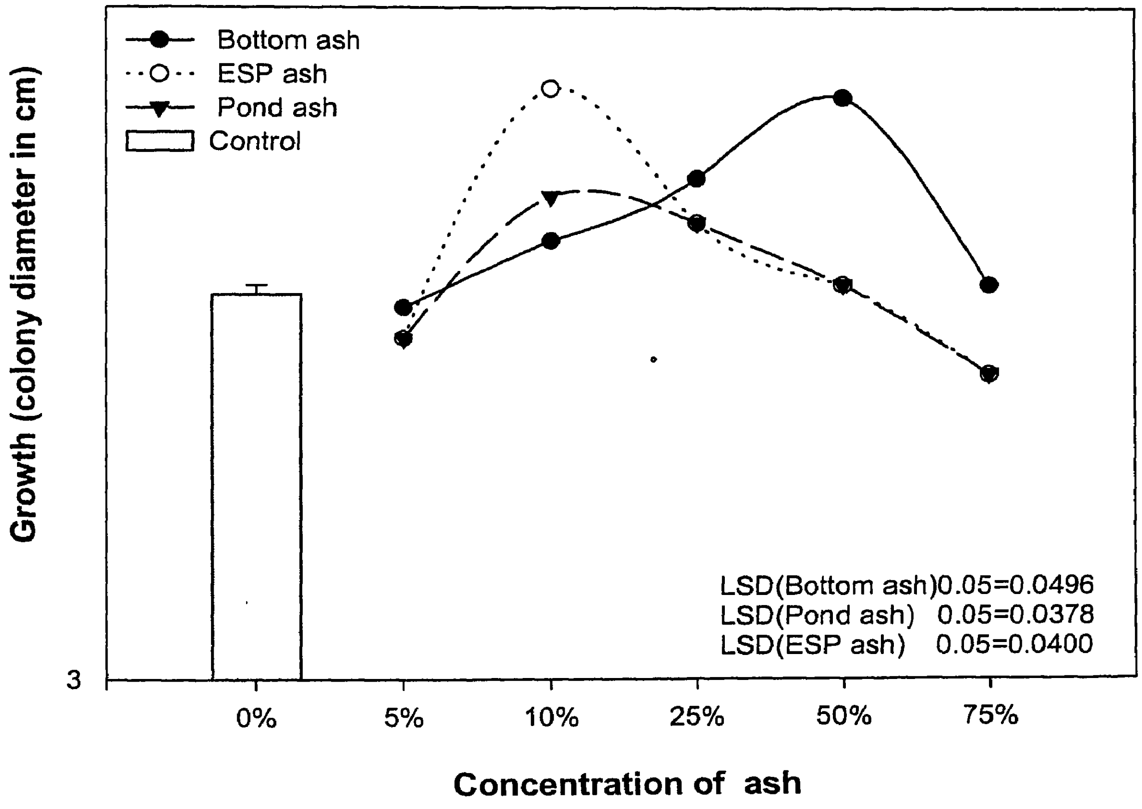


Figure 3.2.2 Growth response of *Pisolithus tinctorius* (EM-1081) at differe concentrations of three types of ashes

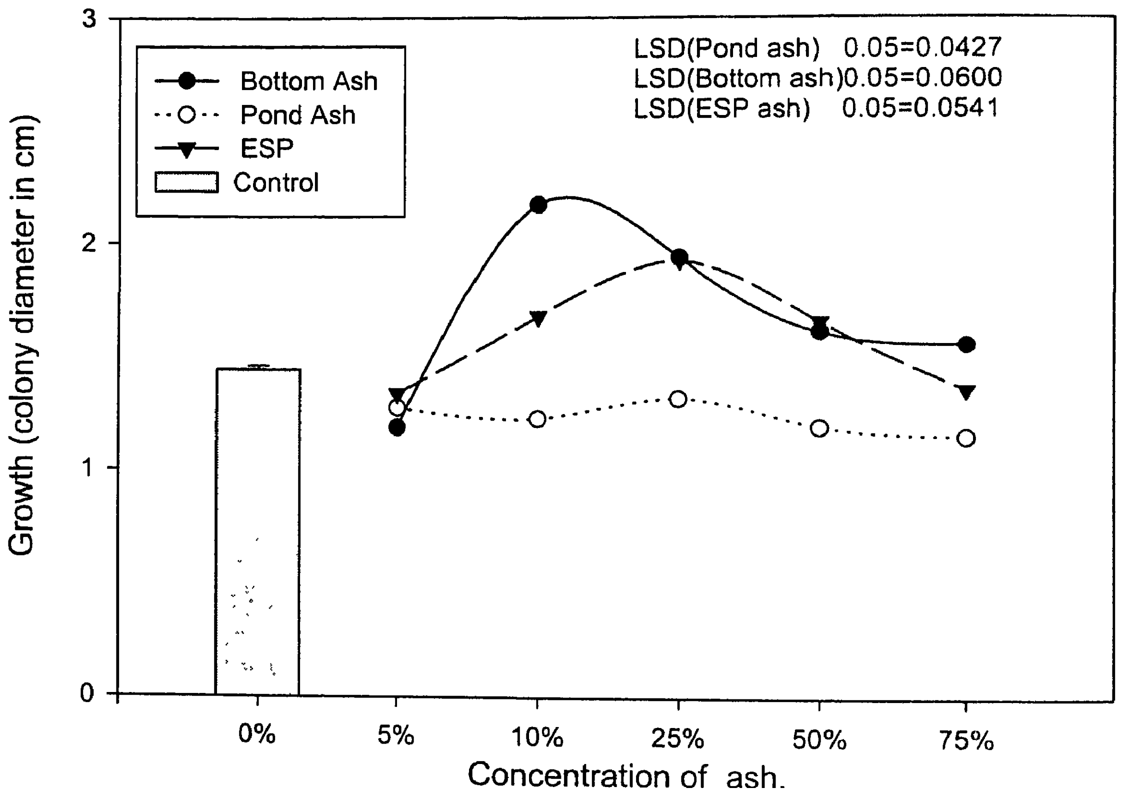
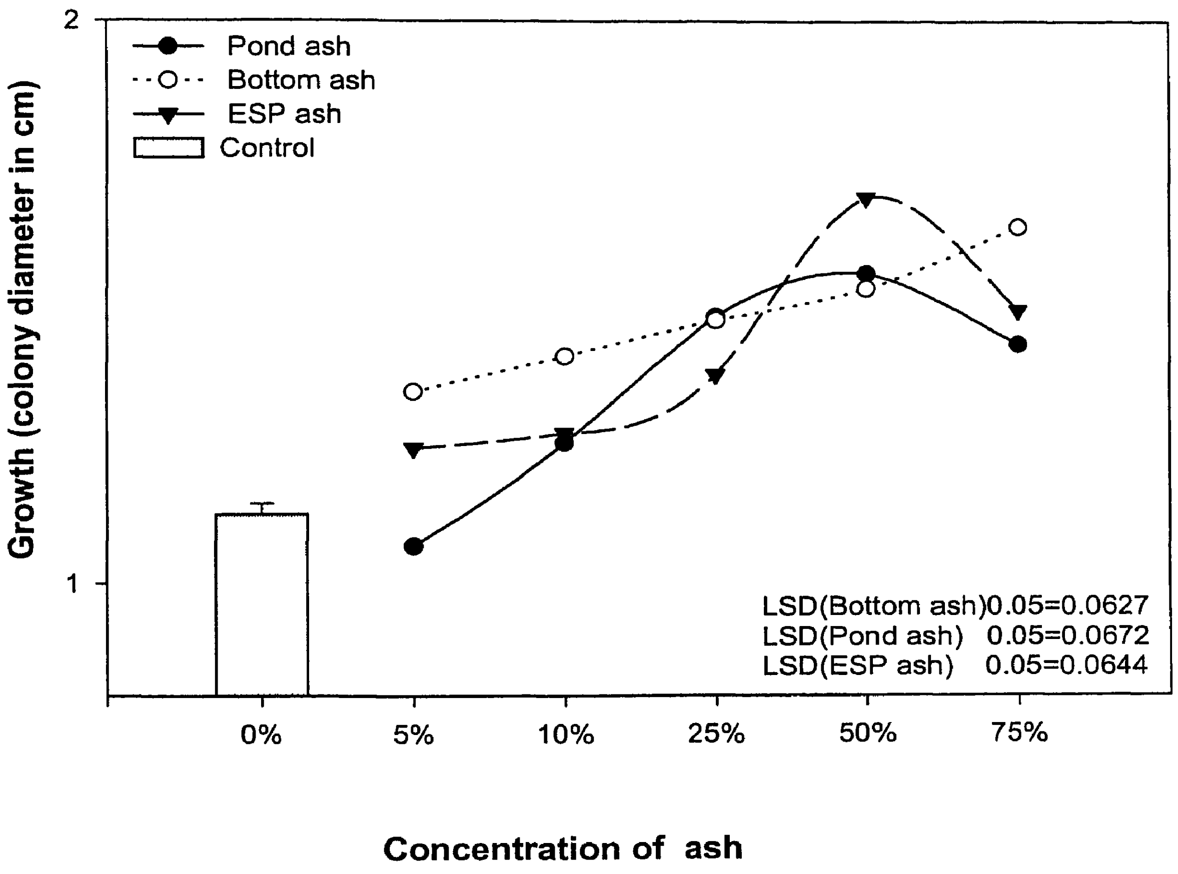


Figure 3.2.3 Growth response of *Pisolithus tinctorius* (EM-1290) at different concentrations of three types of ashes



igure 3.2.6 Growth response of *Pisolithus tinctorius* (EM-1293) at different concentrat of three different types of ashes

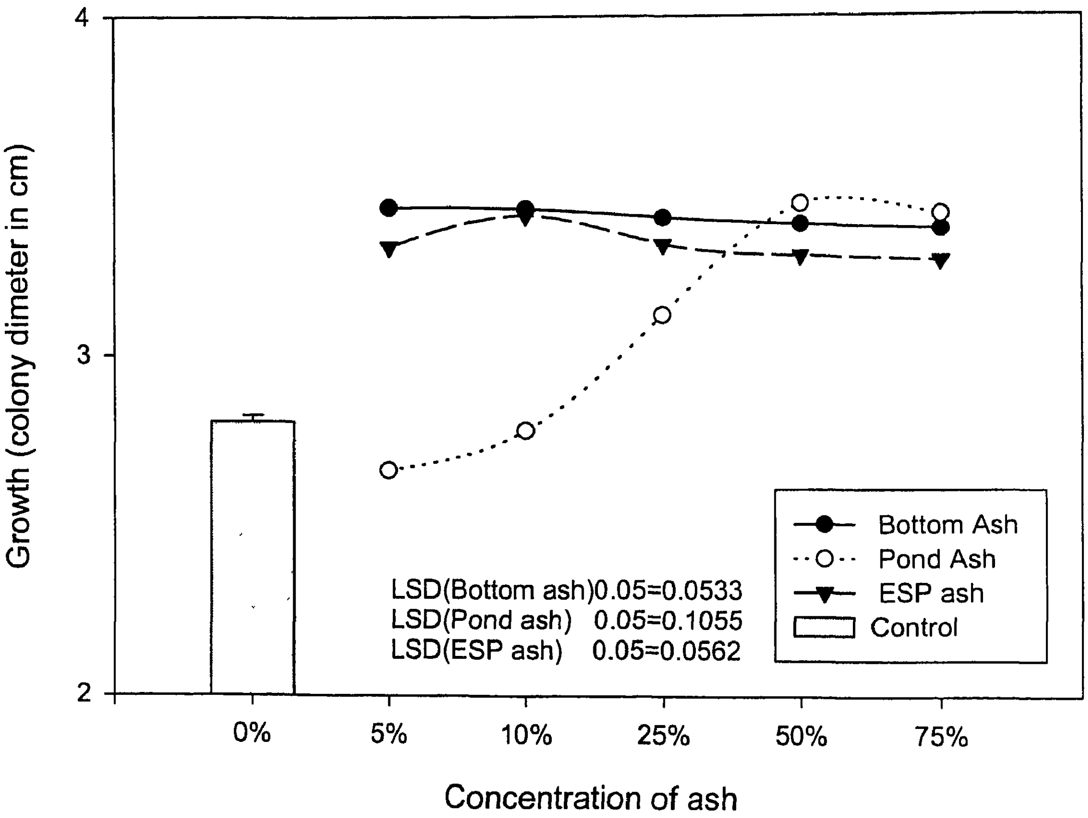


Figure 3.3.1 Growth response and corresponding Aluminium uptake in the mycelia of *Laccaria fraterna* (EM-1083)

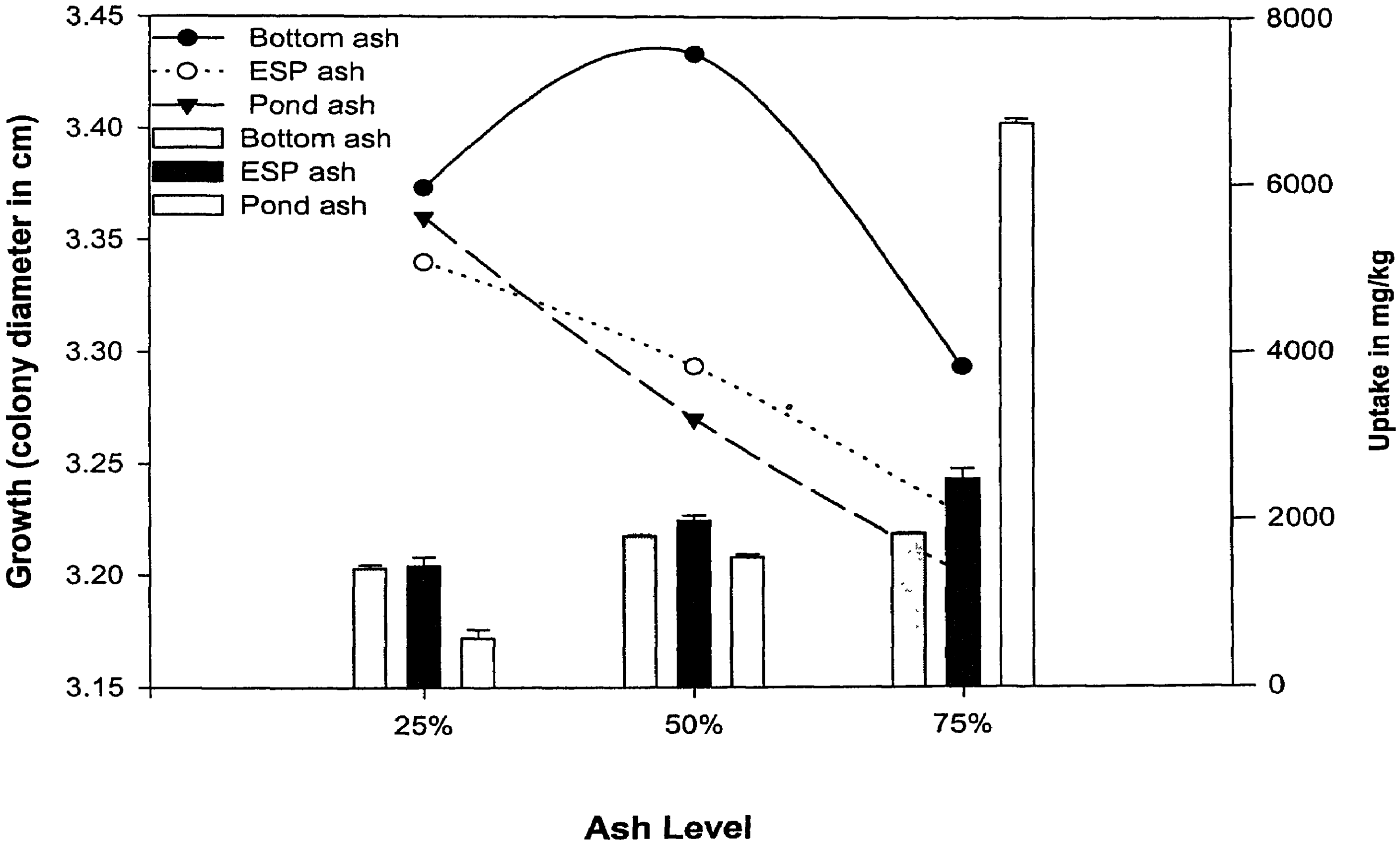


Figure 3.3.2 Growth response and corresponding Nickel uptake in the mycelia o *Laccaria fraterna* (EM-1083)

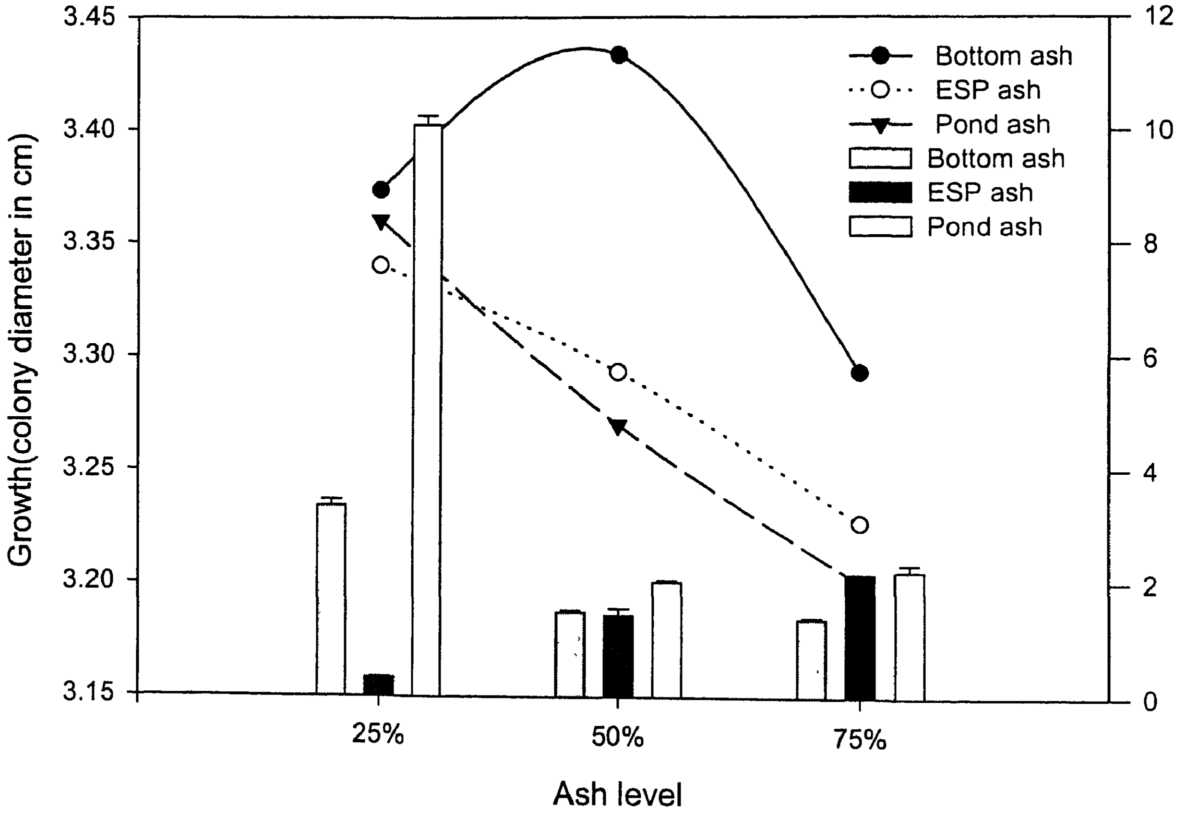


Figure 3.3.3 Growth response and corresponding lead uptake in the mycelia of *Laccaria fraterna* (EM-1083)

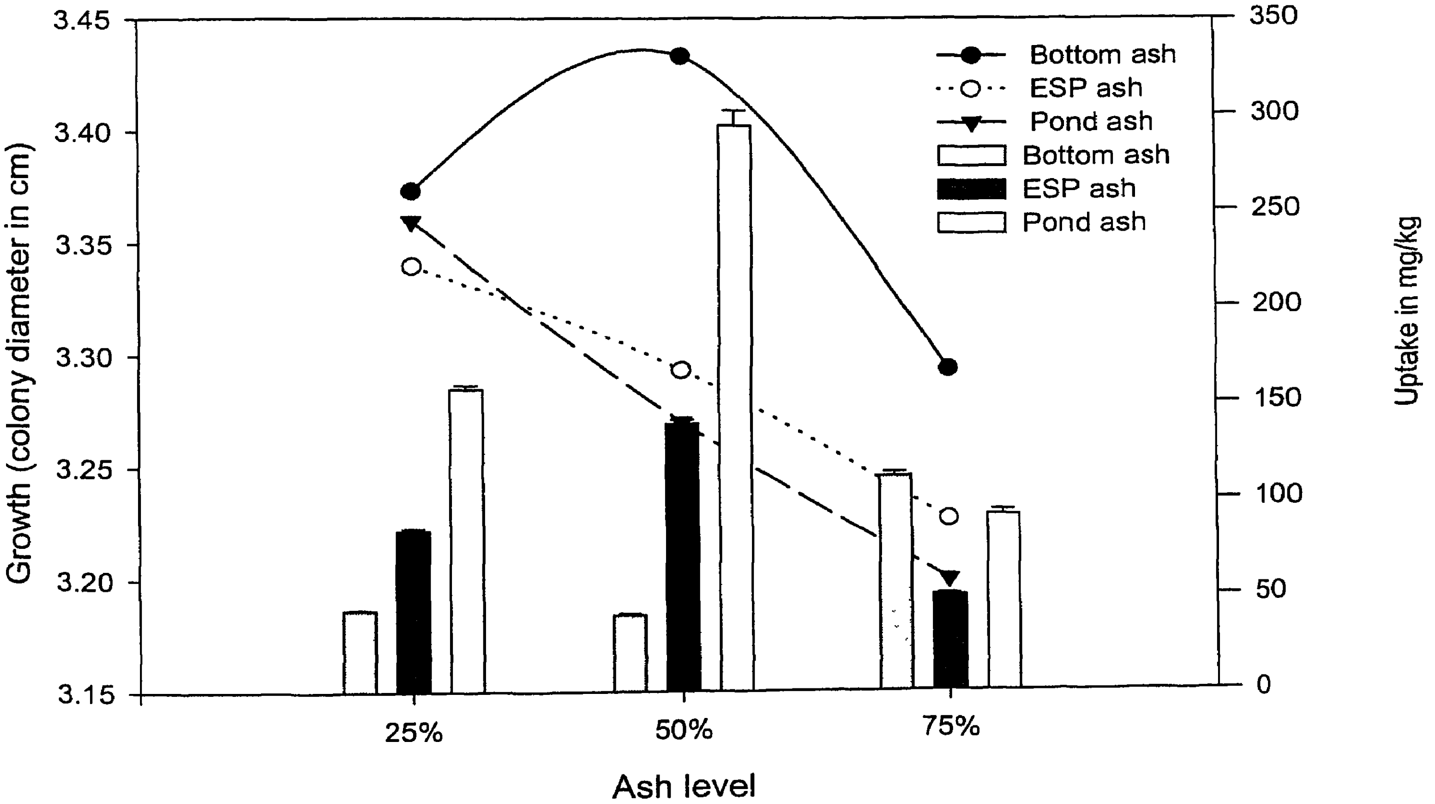


Figure 3.3.4 Growth response and corresponding Chromium uptake in the mycelia of *Laccaria fraterna* (EM-1083)

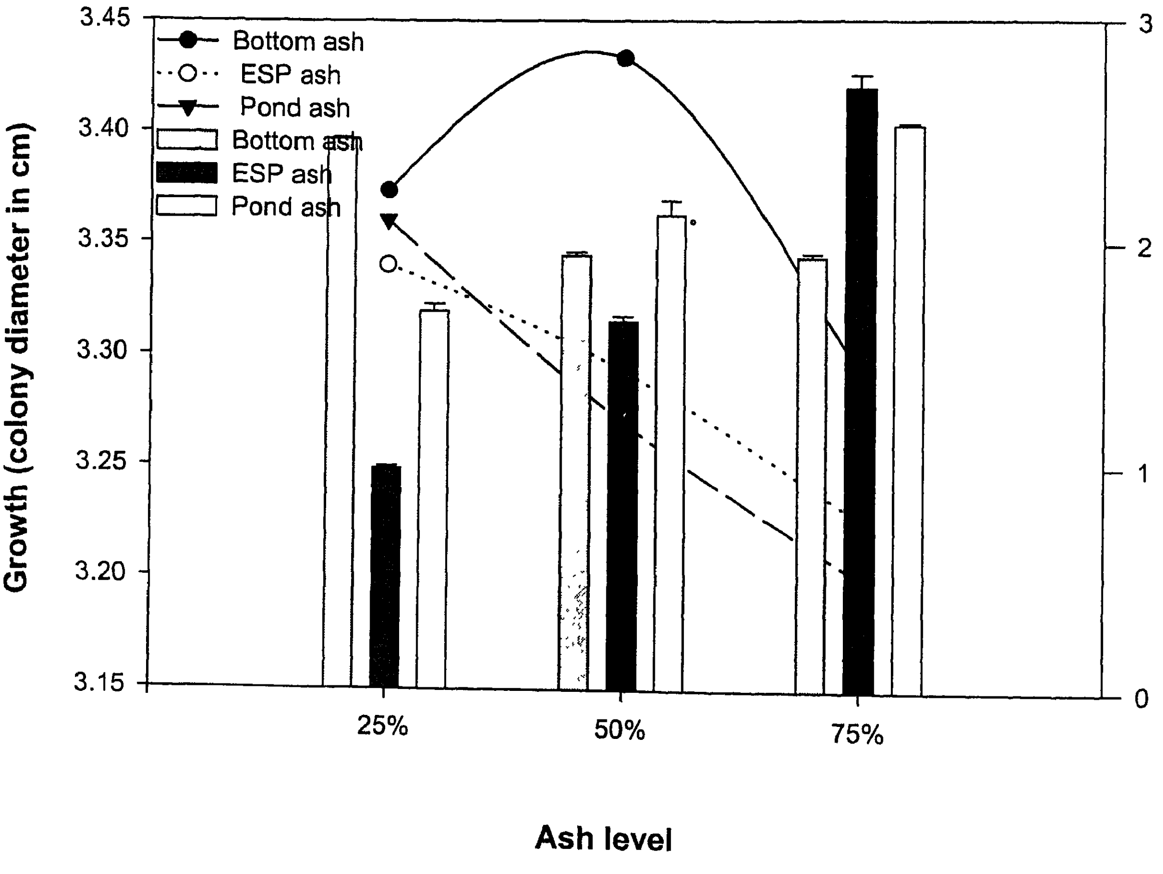


Figure 3.3.5 Growth response and corresponding cadmium uptake in the mycelia *Laccaria fraterna* (EM-1083)

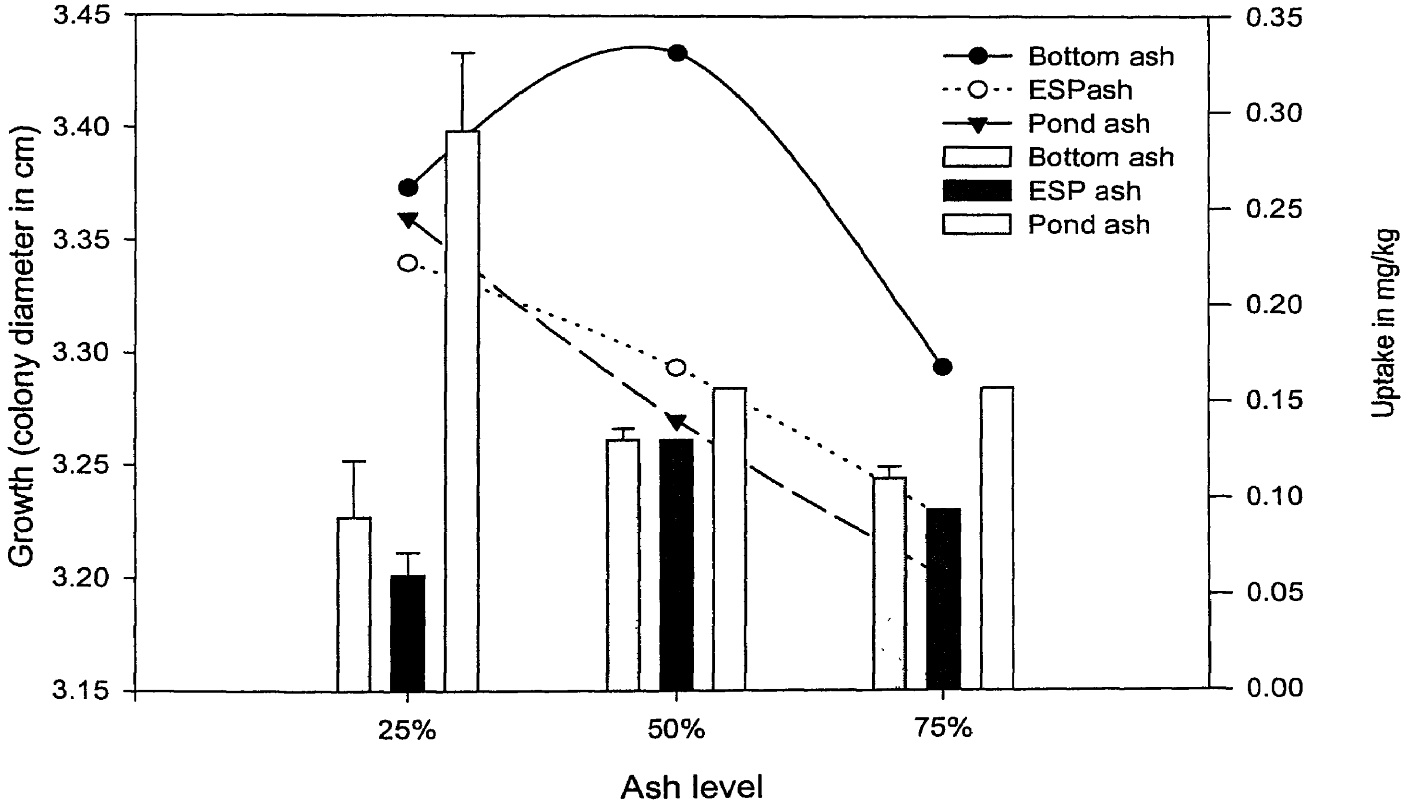


Figure 3.3.6 Growth response and corresponding iron uptake in the mycelia
Laccaria fraterna (EM-1083)

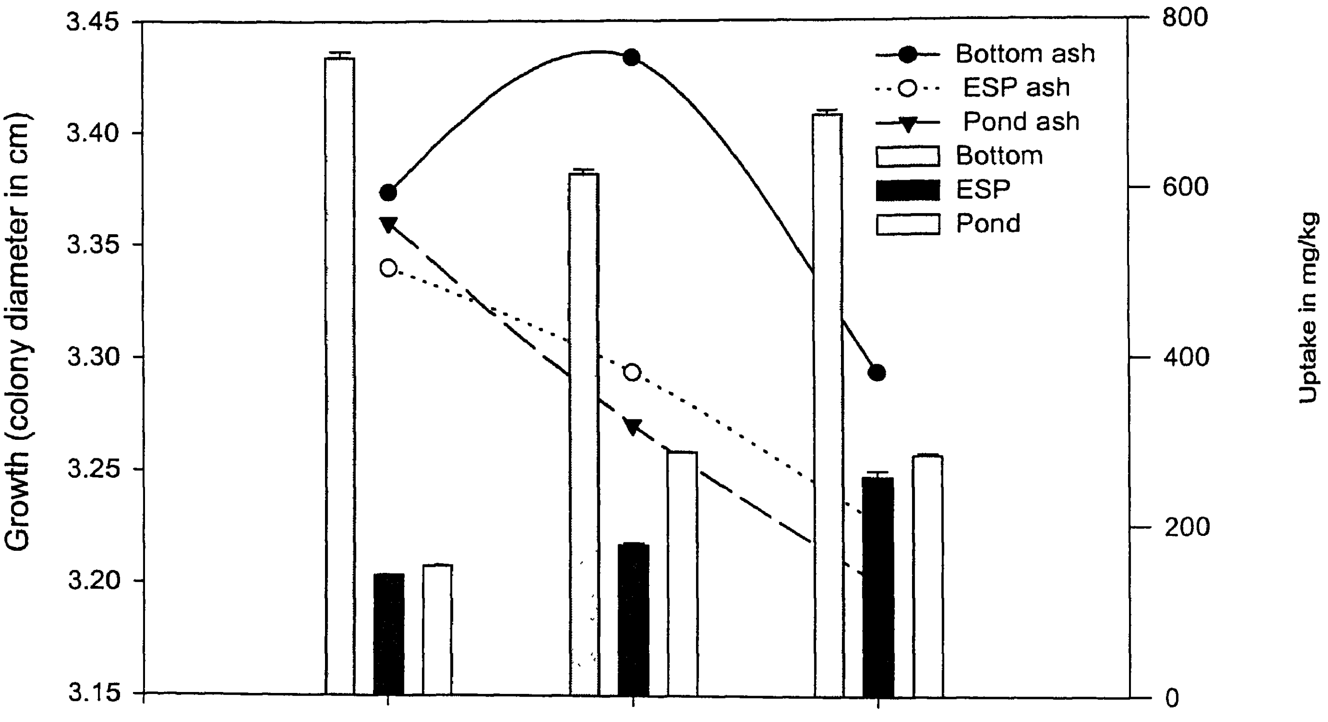


Figure 3.4.1Growth response and coresponding aluminium uptake in the mycelia of *Pisolithus tinctorius* (EM-1081)

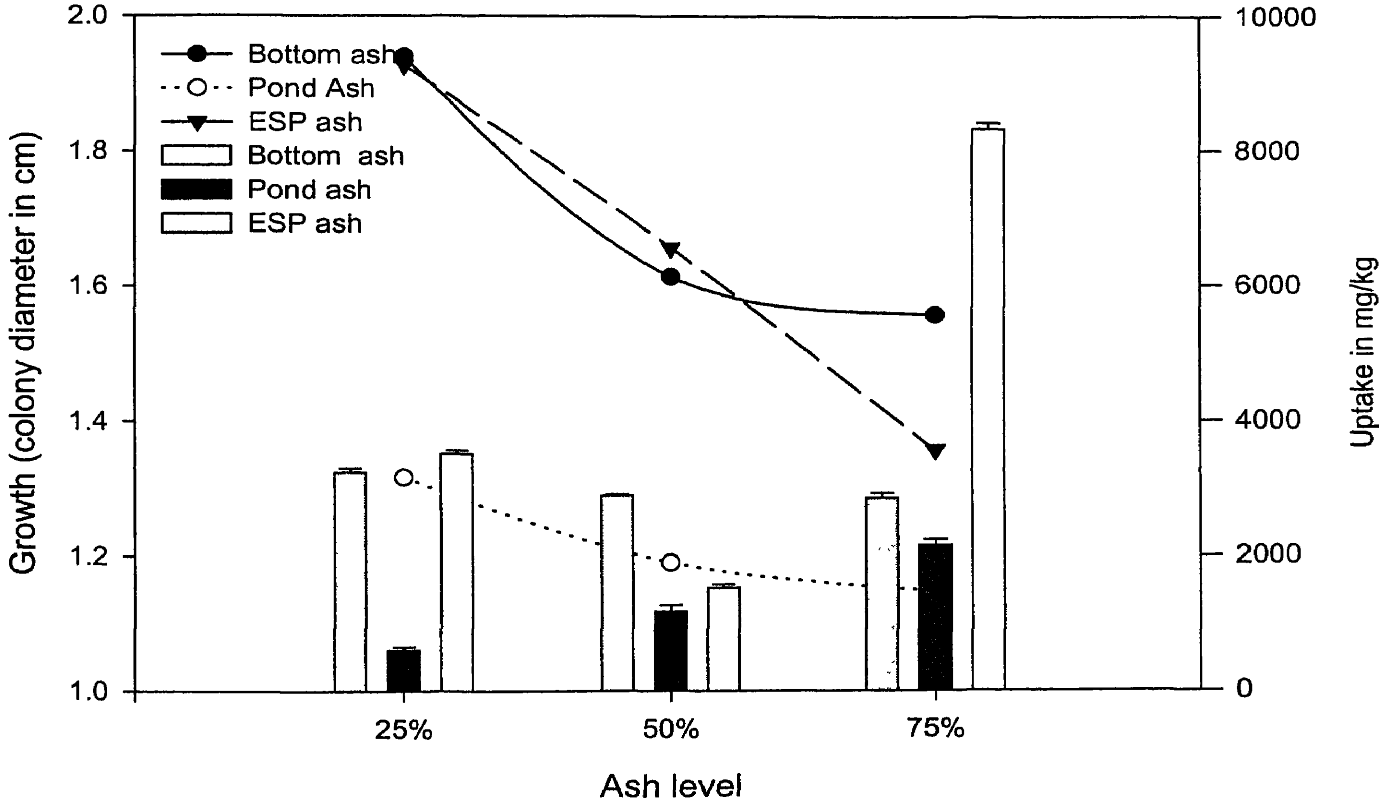


Figure 3.4.2 Growth response and coresponding nickel uptake in the mycelia of *Pisolithus tinctorius* (EM-1081)

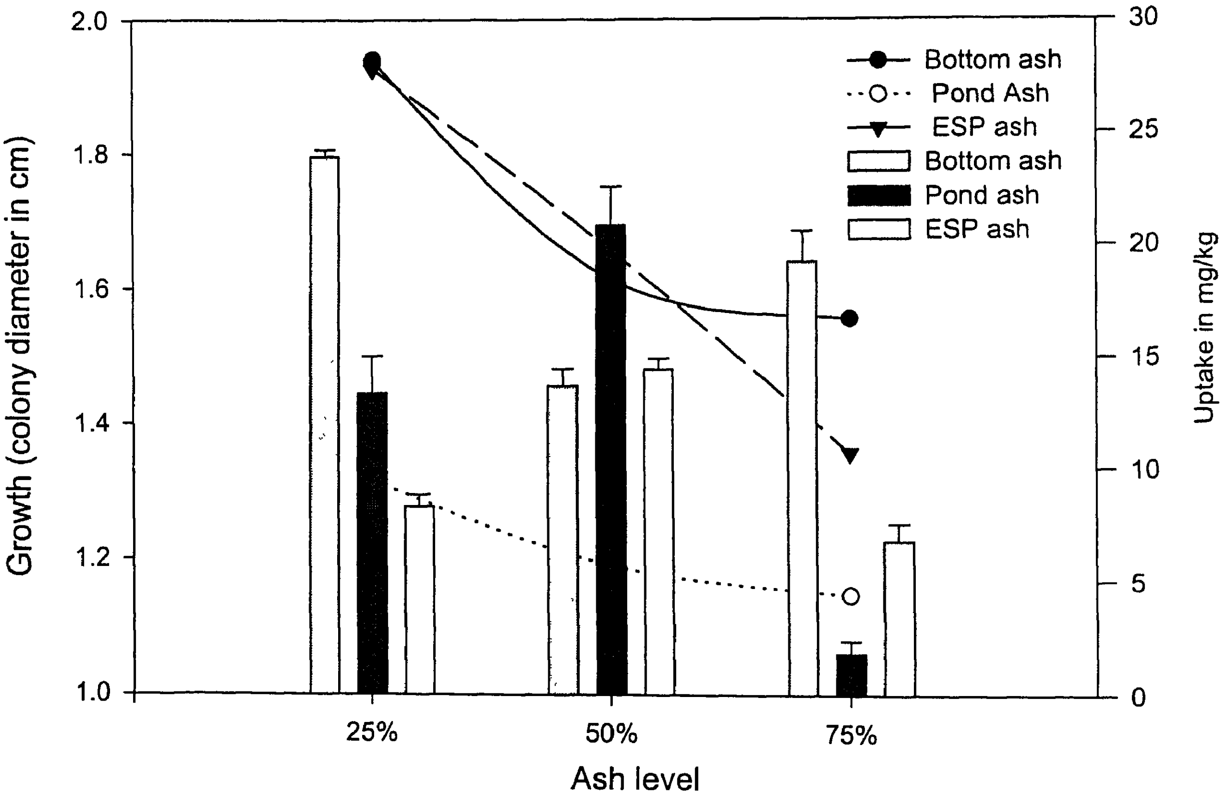


Figure 3.4.3 Growth response and corresponding lead uptake in the mycelia o *Pisolithus tinctorius* (EM-1081)

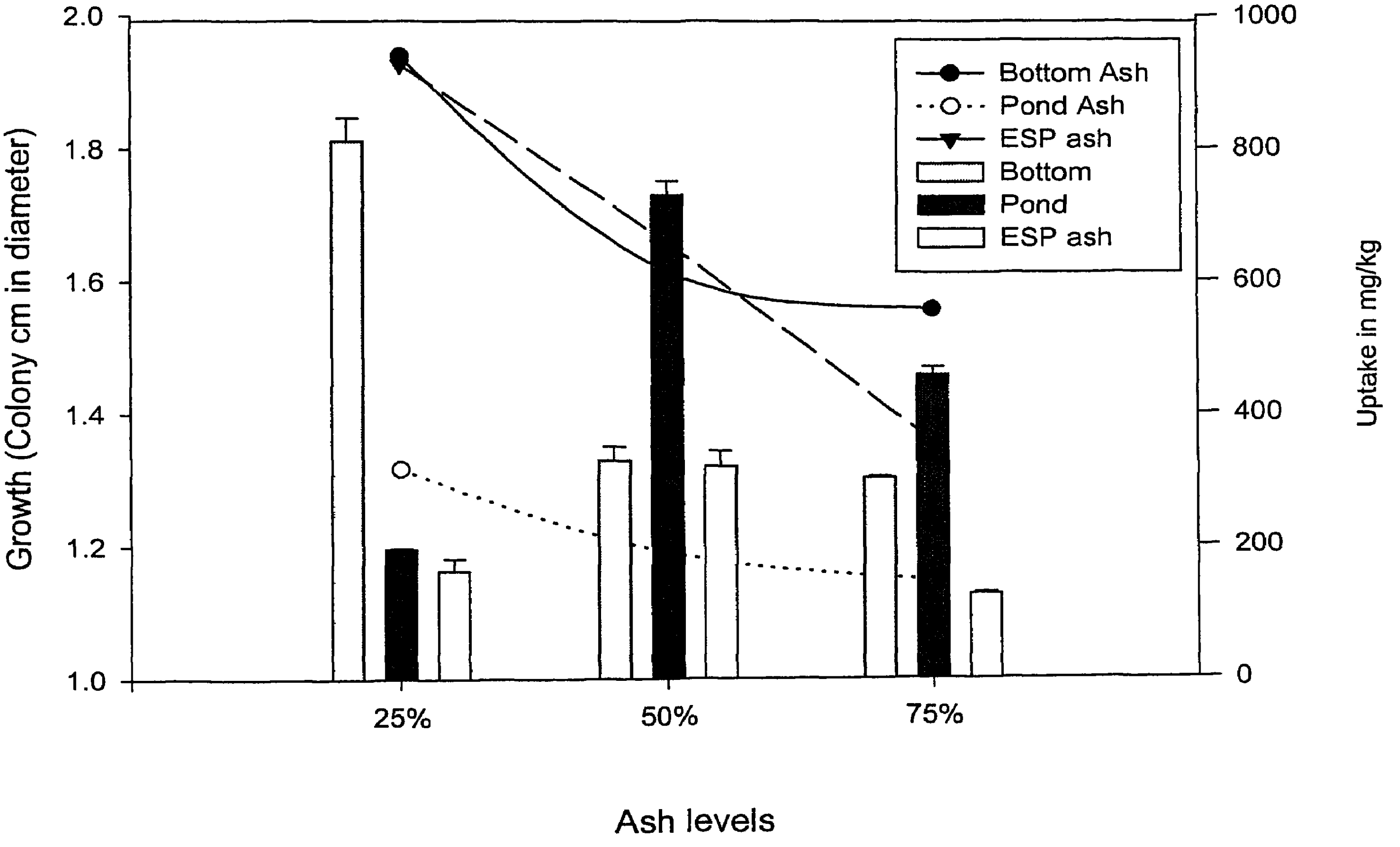


Figure 3.4.4 Growth response and corresponding chromium uptake in the mycelia of *Pisolithus tinctorius* (EM-1081)

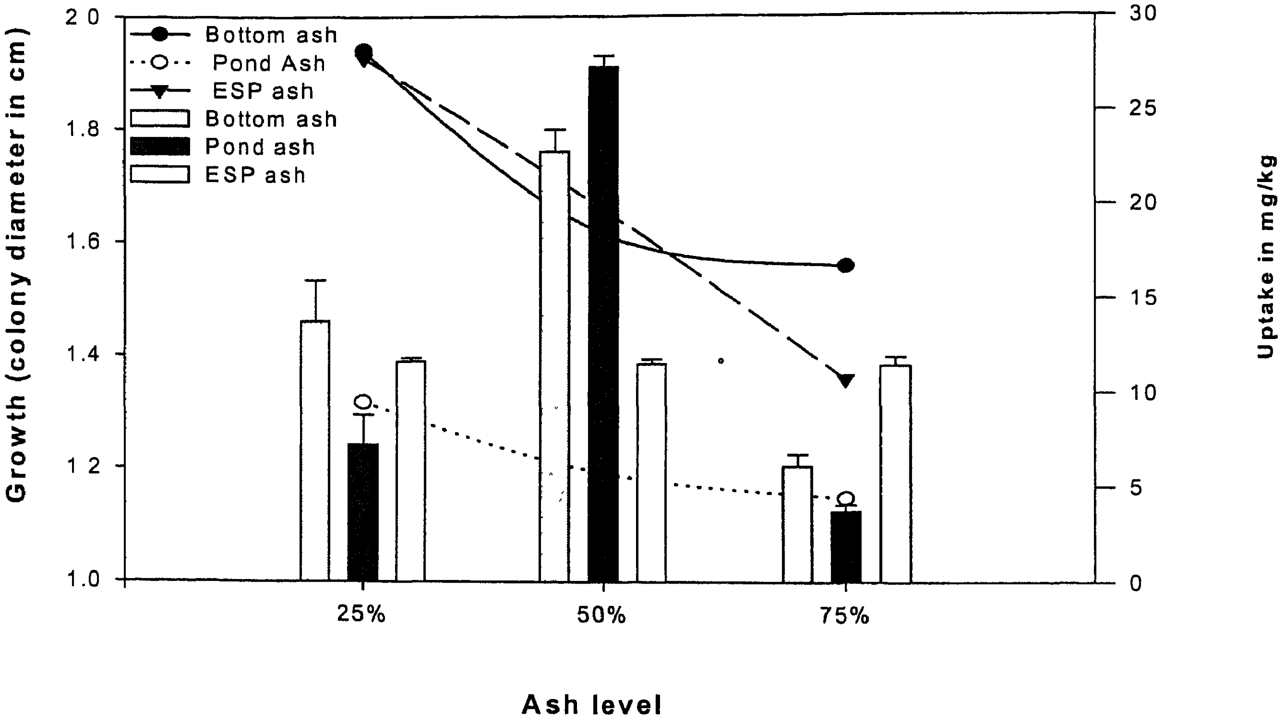


Figure 3.4.5 Growth response and corresponding cadmium uptake in the mycelia o *Pisolithus tinctorius* (EM-1081)

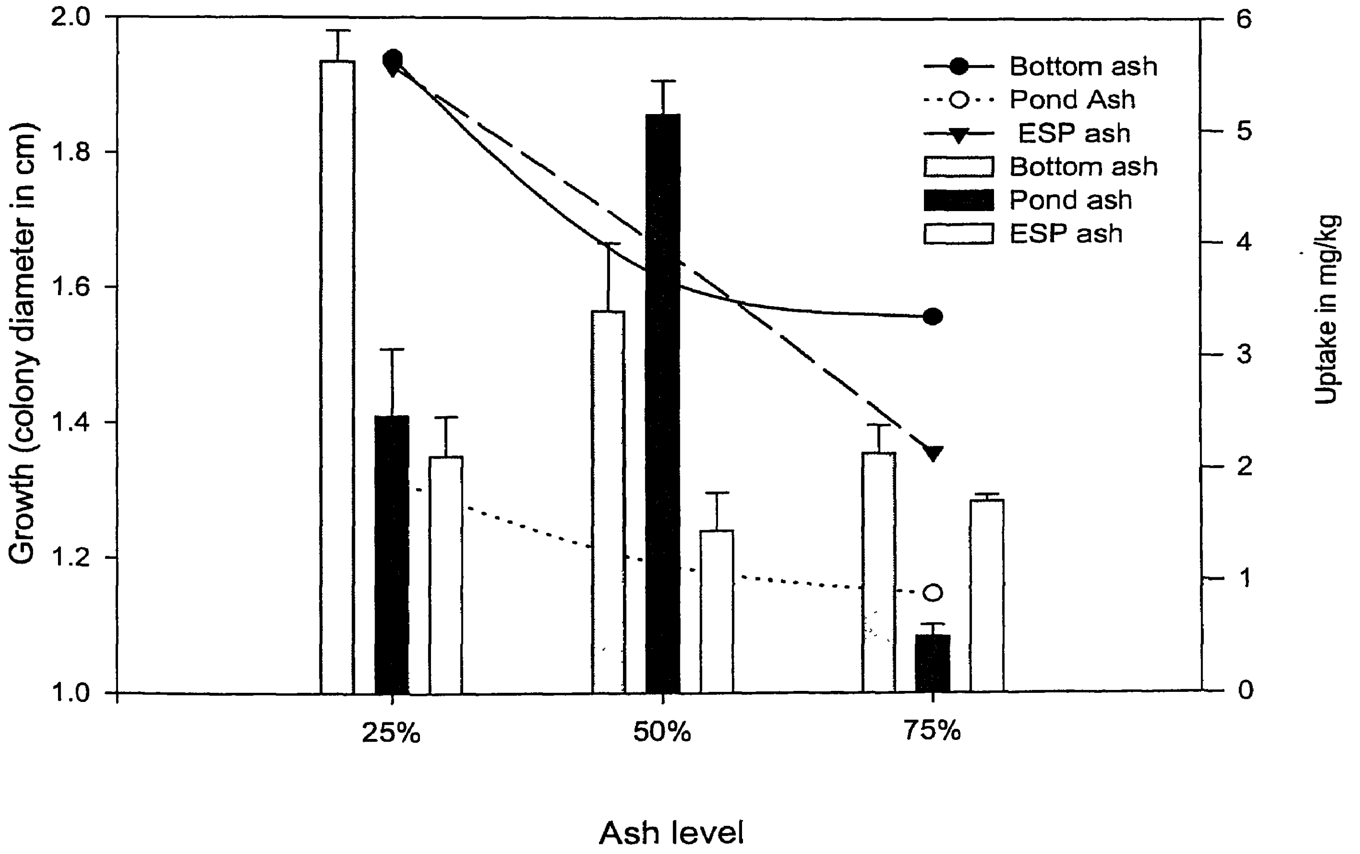


Figure 3.4.6 Growth response and corresponding iron uptake in the mycelia of *Pisolithus tinctorius* (EM-1081)

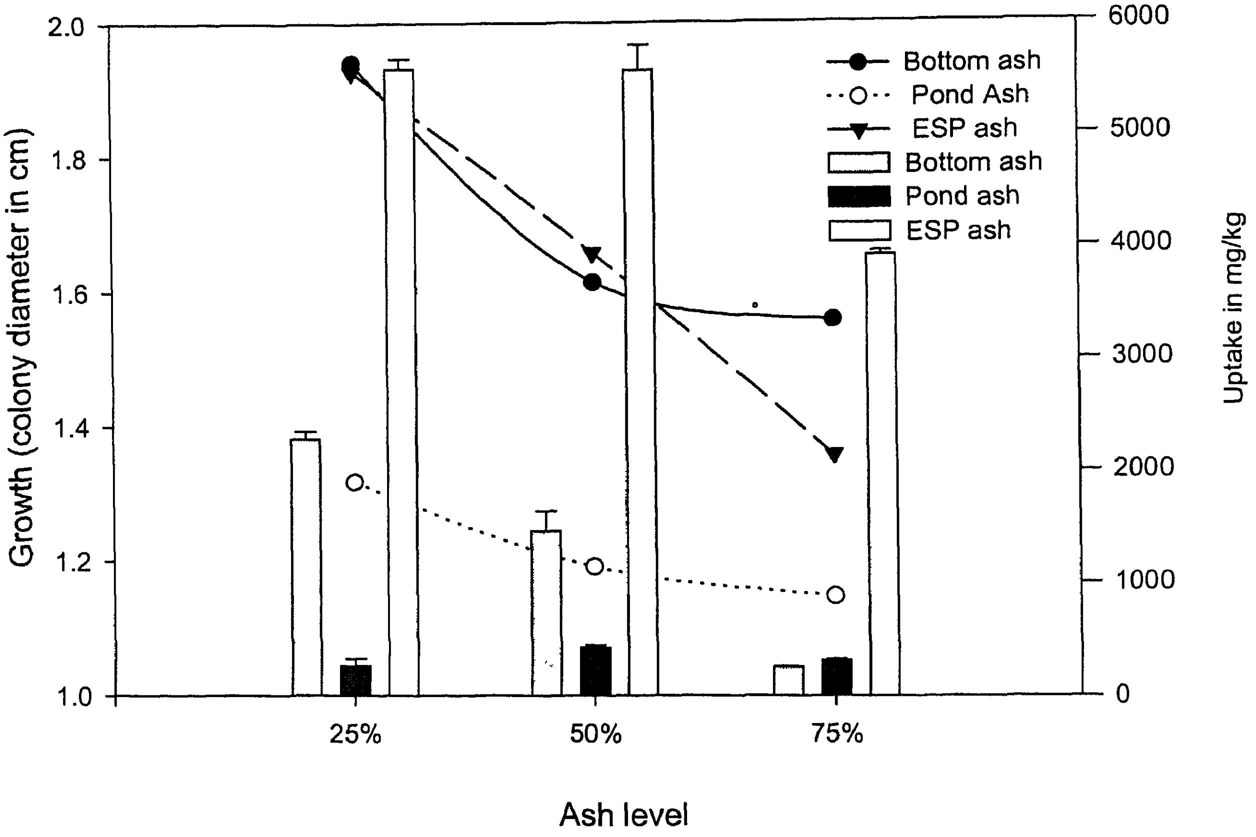


Figure 3.5.1 Growth response and corresponding aluminium uptake in the mycelia o *Pisolithus tinctorius* (EM-1290)

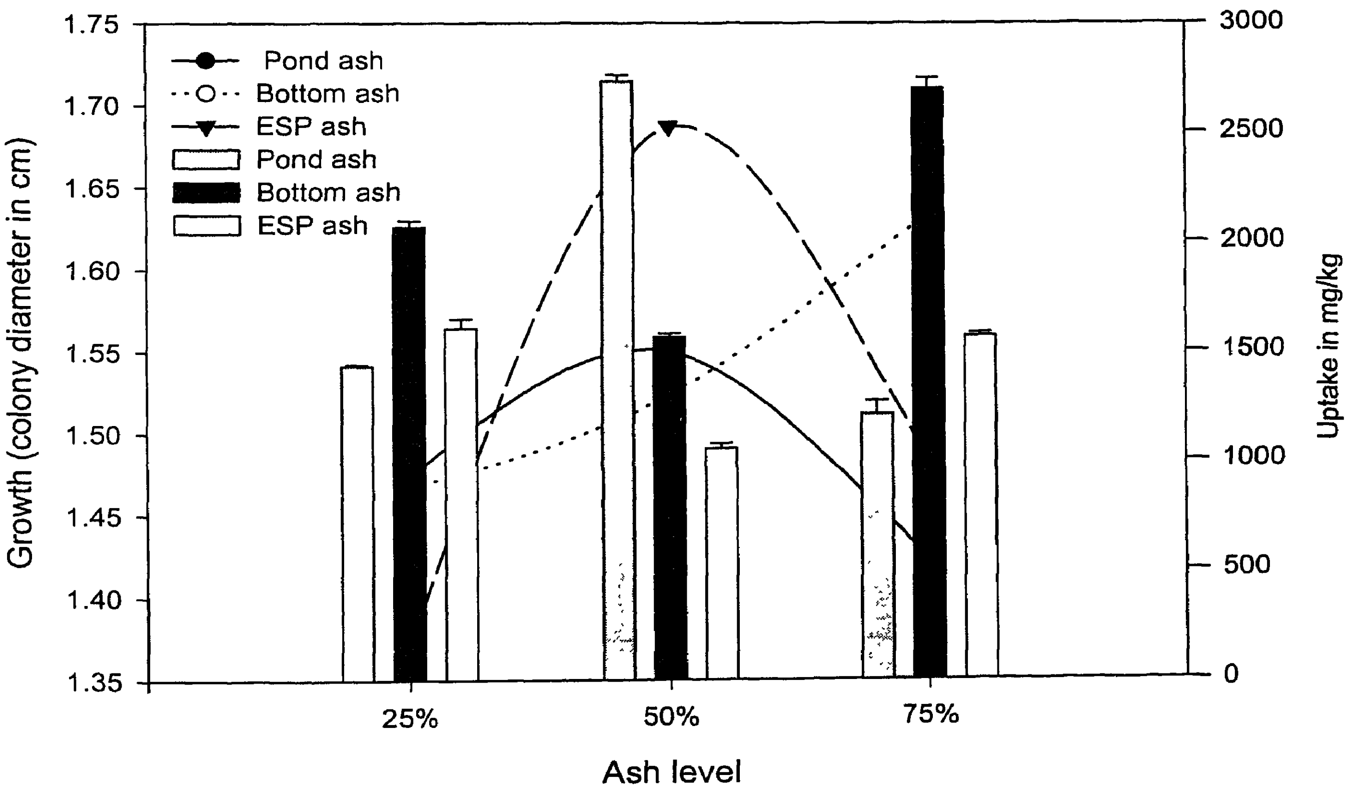


Figure 3.5.2 Growth response and corresponding nickel uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)

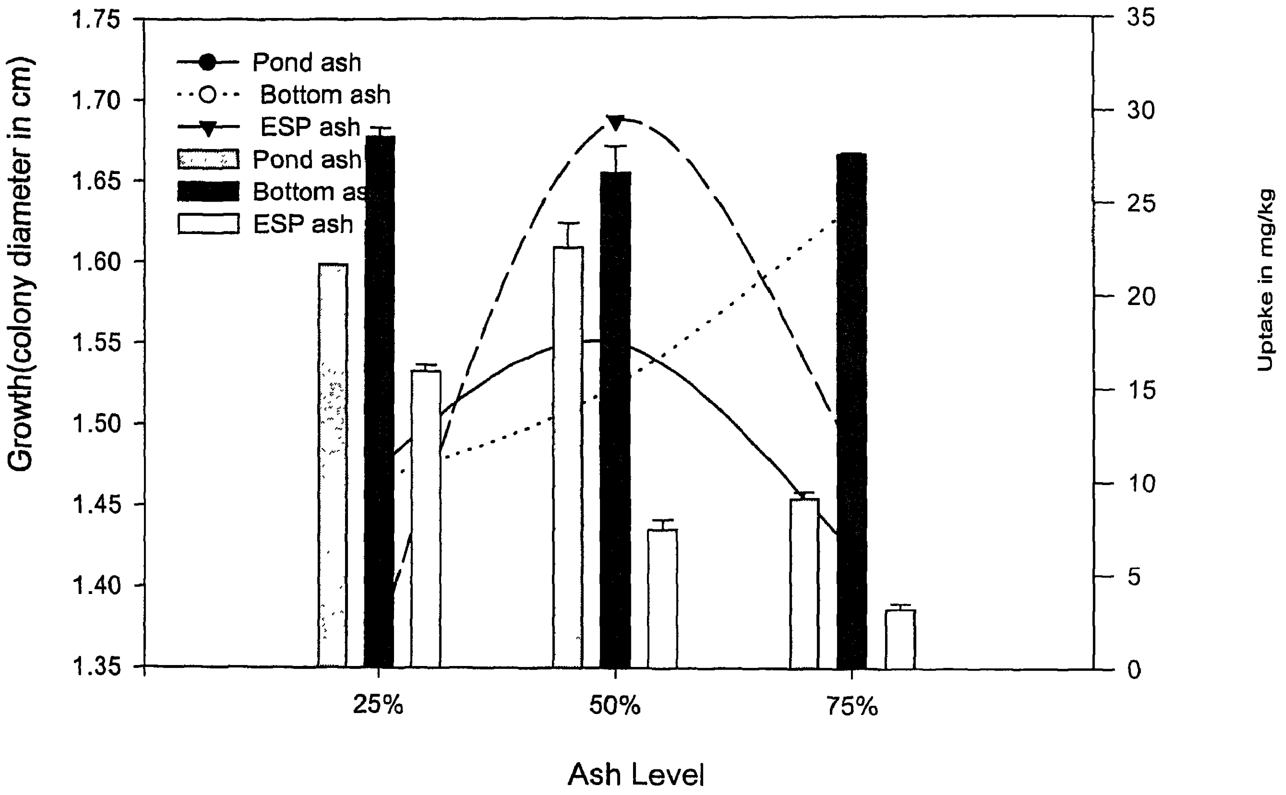


Figure3.5.3 Growth response and corresponding lead uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)

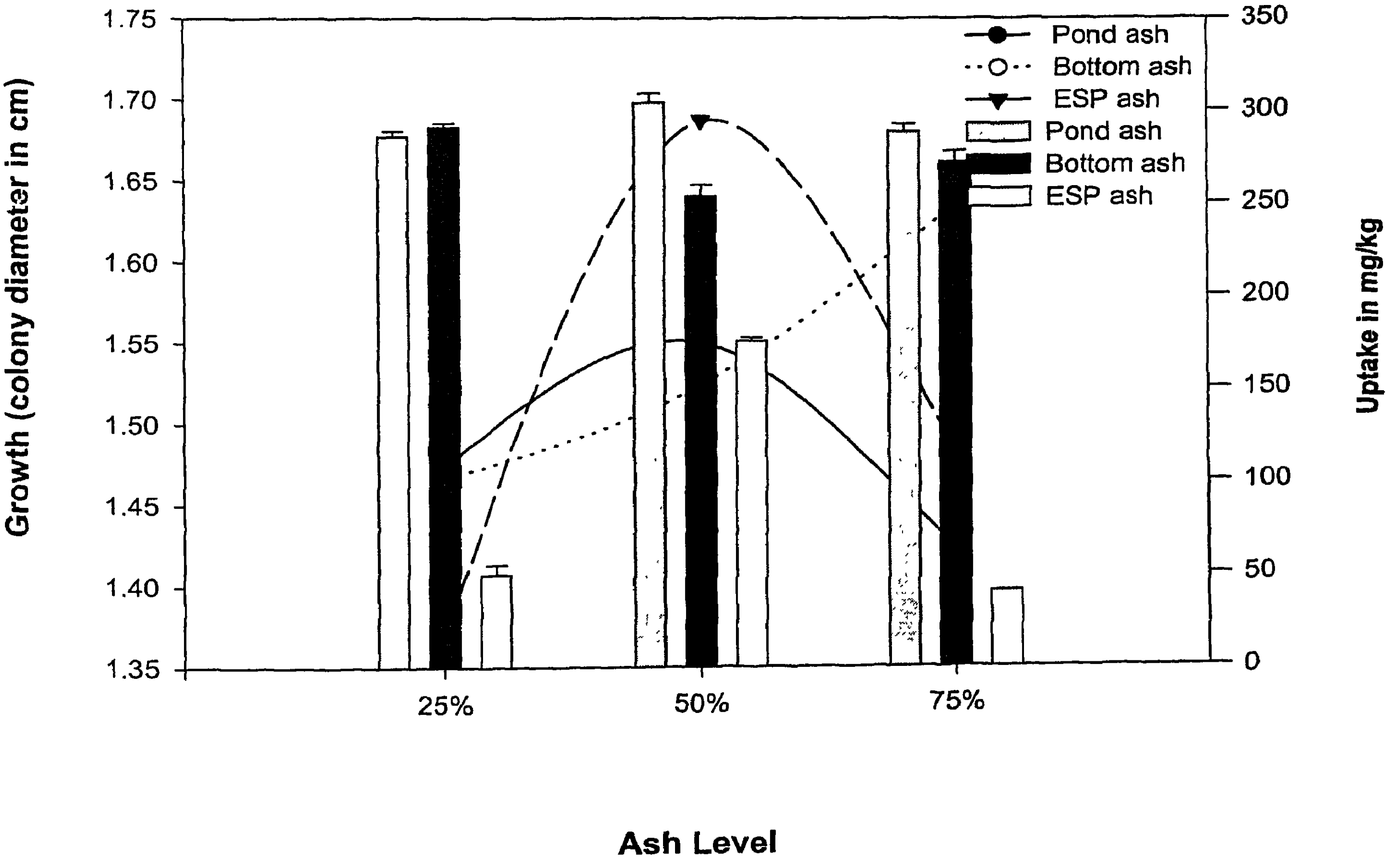


Figure 3.5.4 Growth response and corresponding chromium uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)

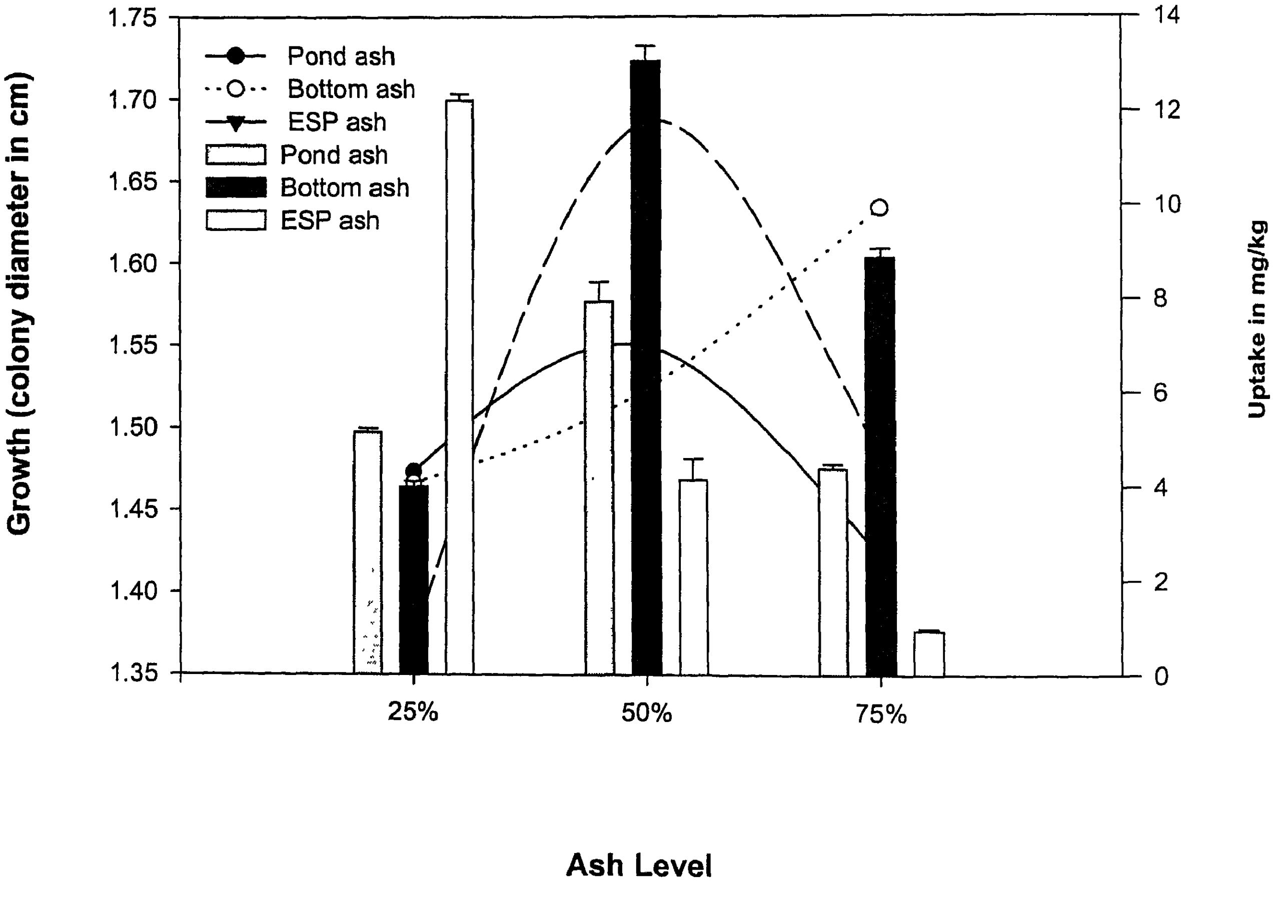


Figure 3.5.5 Growth response and corresponding cadmium uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)

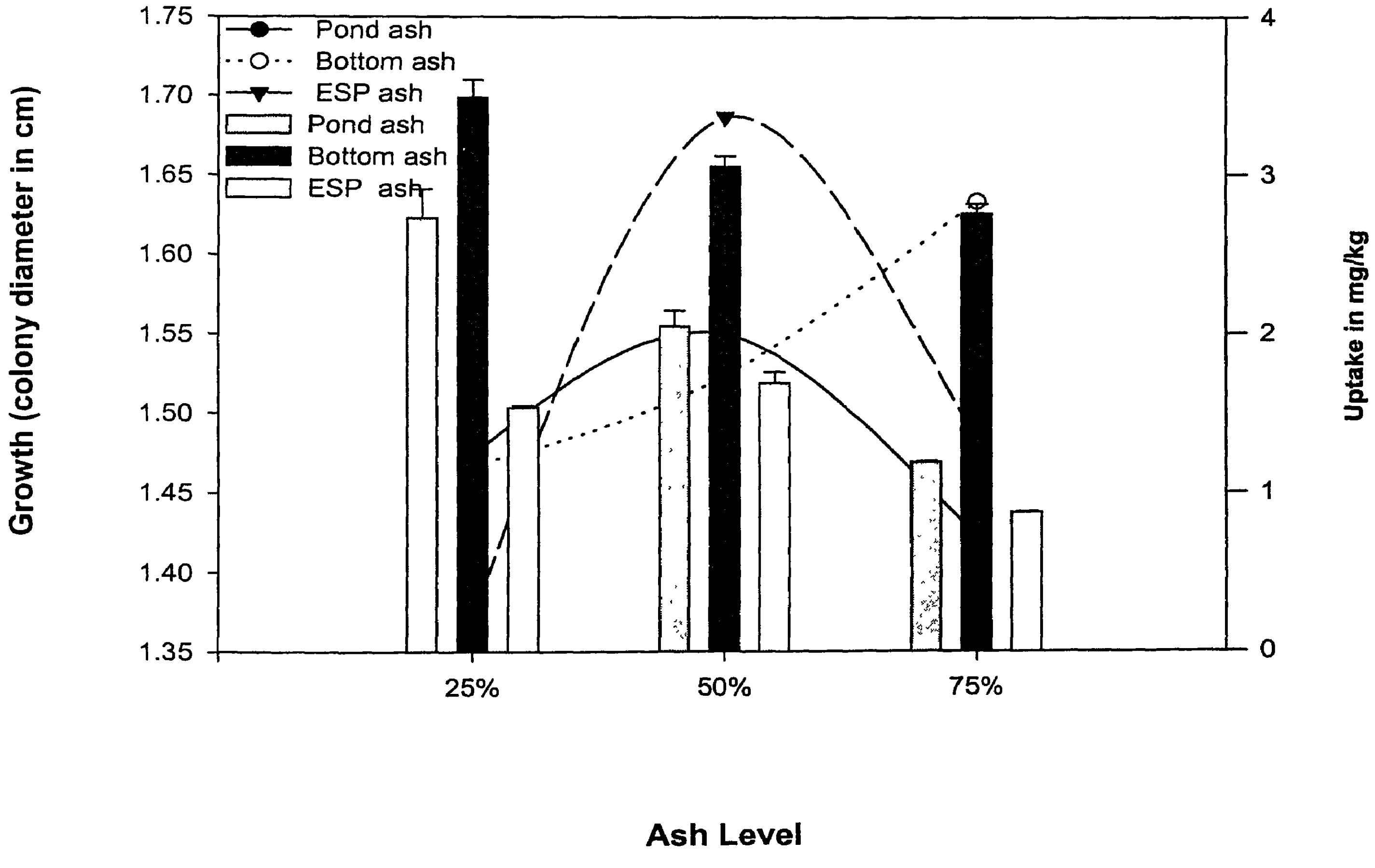


Figure 3.5.6 Growth response and corresponding iron uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)

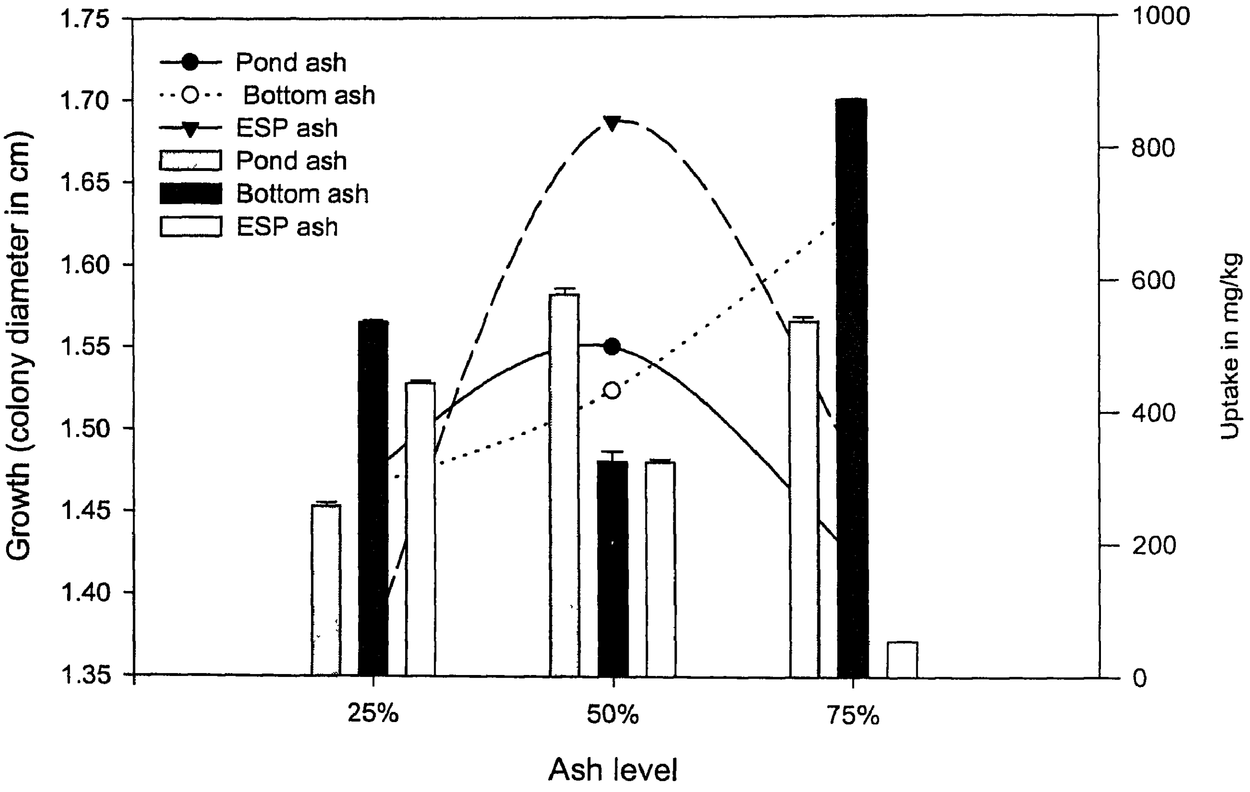


Figure 3.6.1 Growth response and corresponding aluminium uptake in the mycelia c of *Pisolithus tinctorius* (EM-1293)

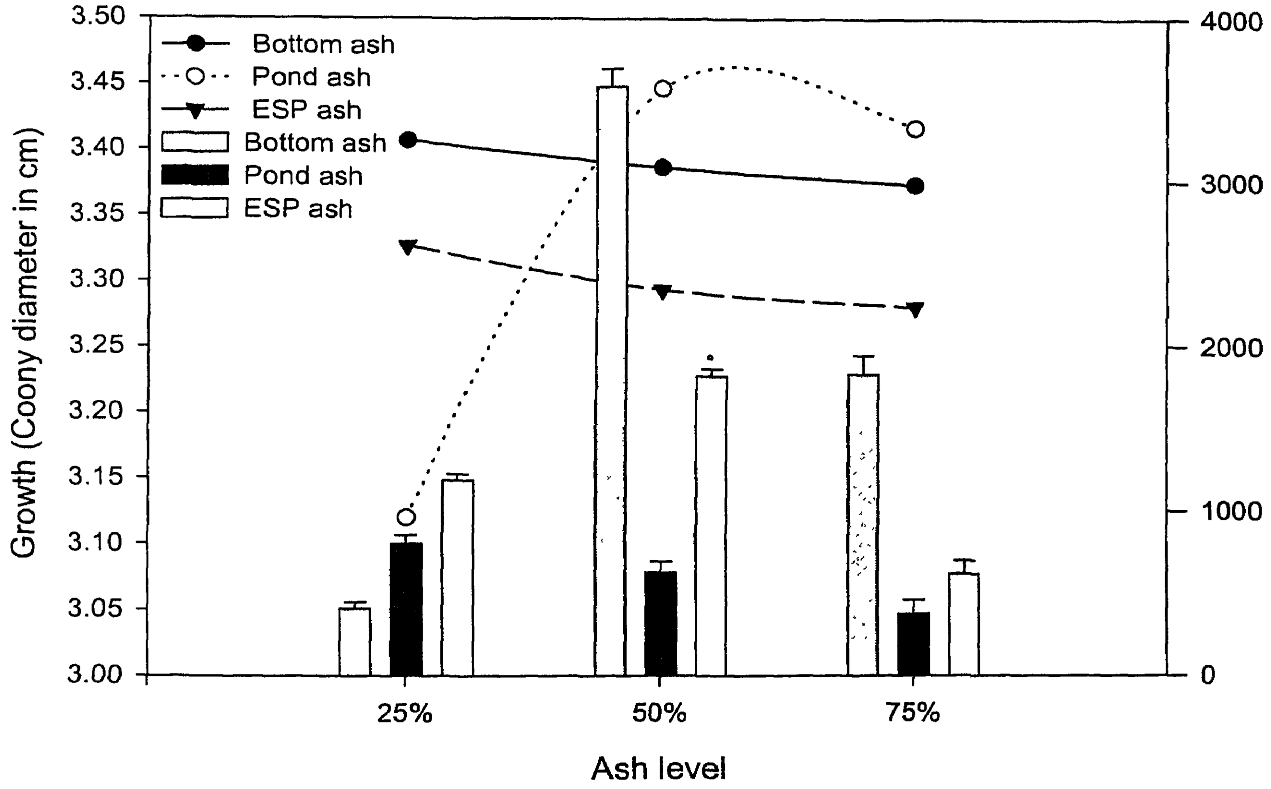


Figure 3.6.2 Growth response and corresponding nickel uptake in the mycelia of *Pisolithus tInctorius* (EM-1293)

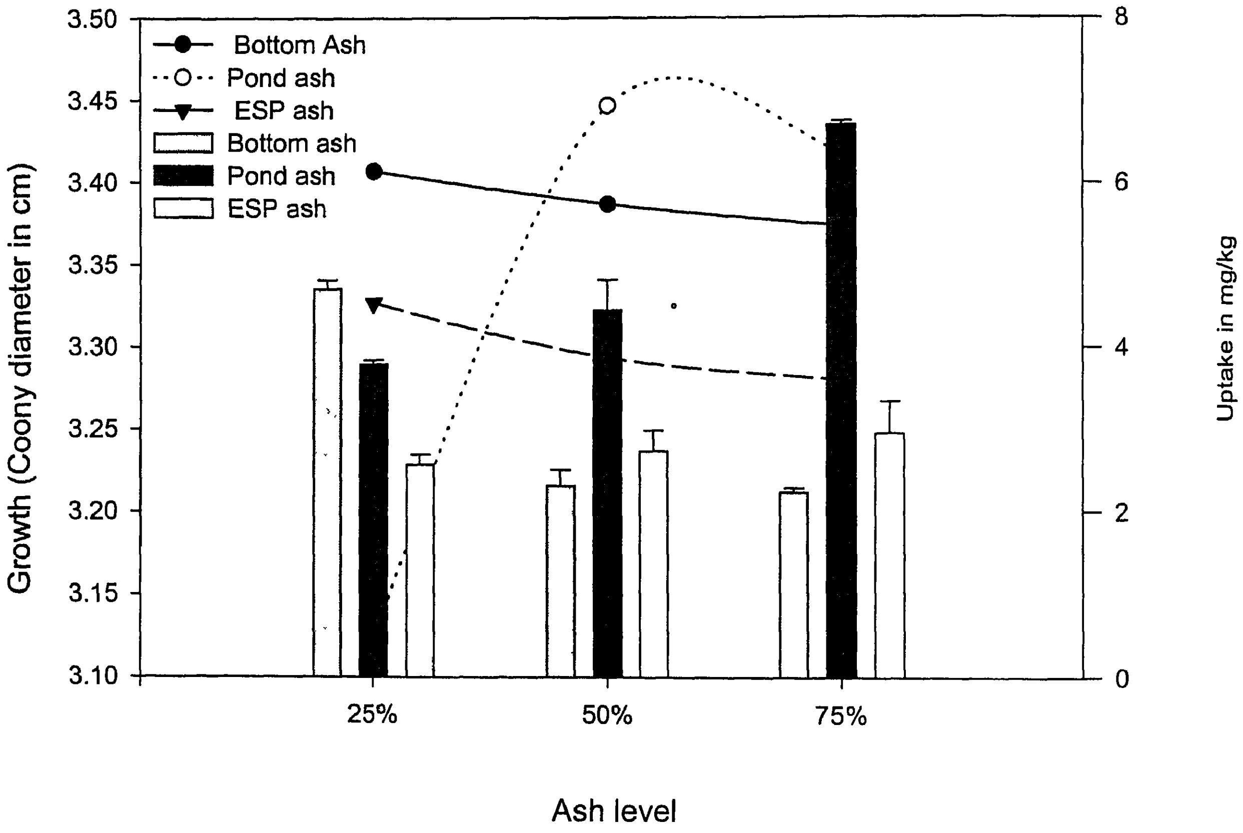
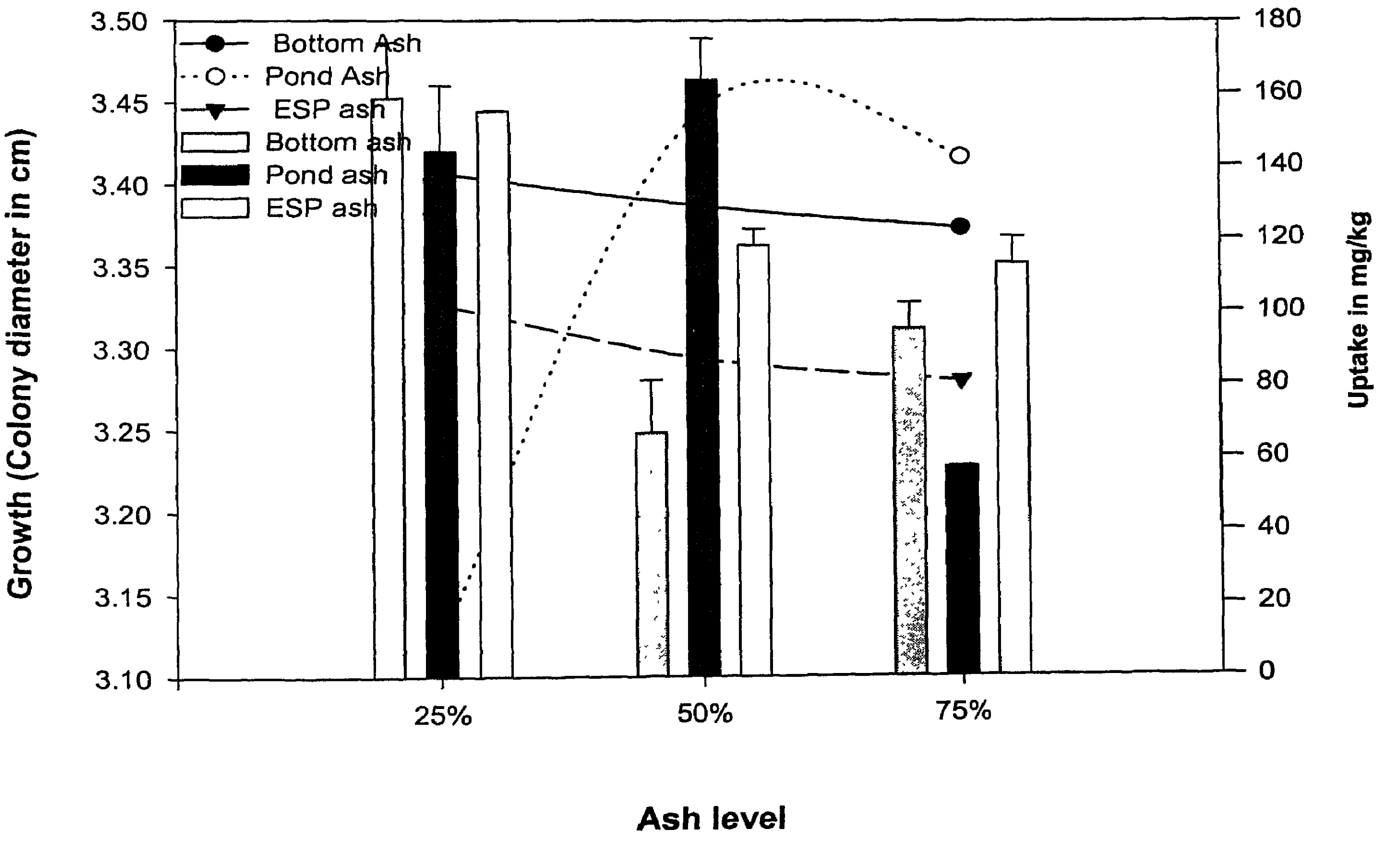


Figure 3.6.3 Growth response and corresponding lead uptake in the mycelia of *Pisolithus tinctorius* (EM-1293)



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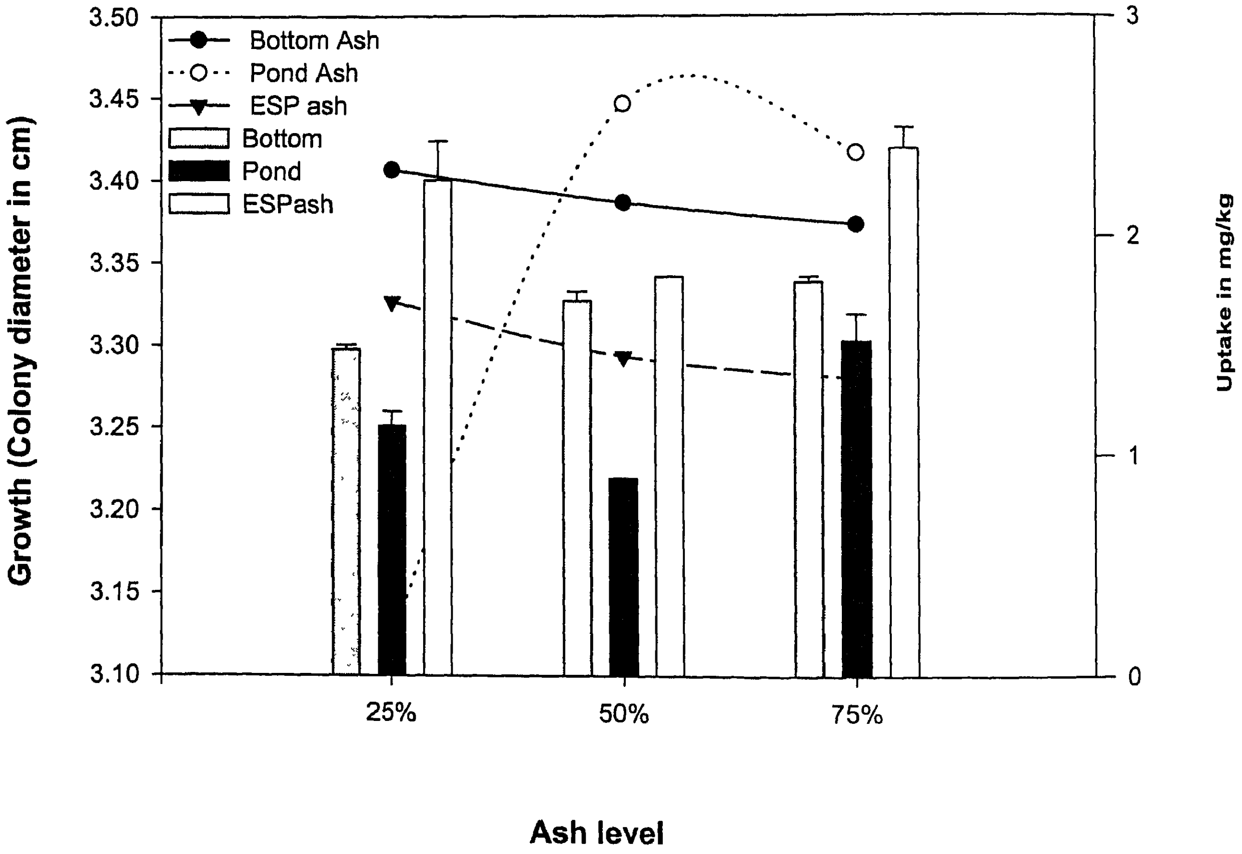


Figure 3.6.5 Growth response and corresponding cadmium uptake in the mycelia of *Pisolithus tinctorius* (EM-1293)

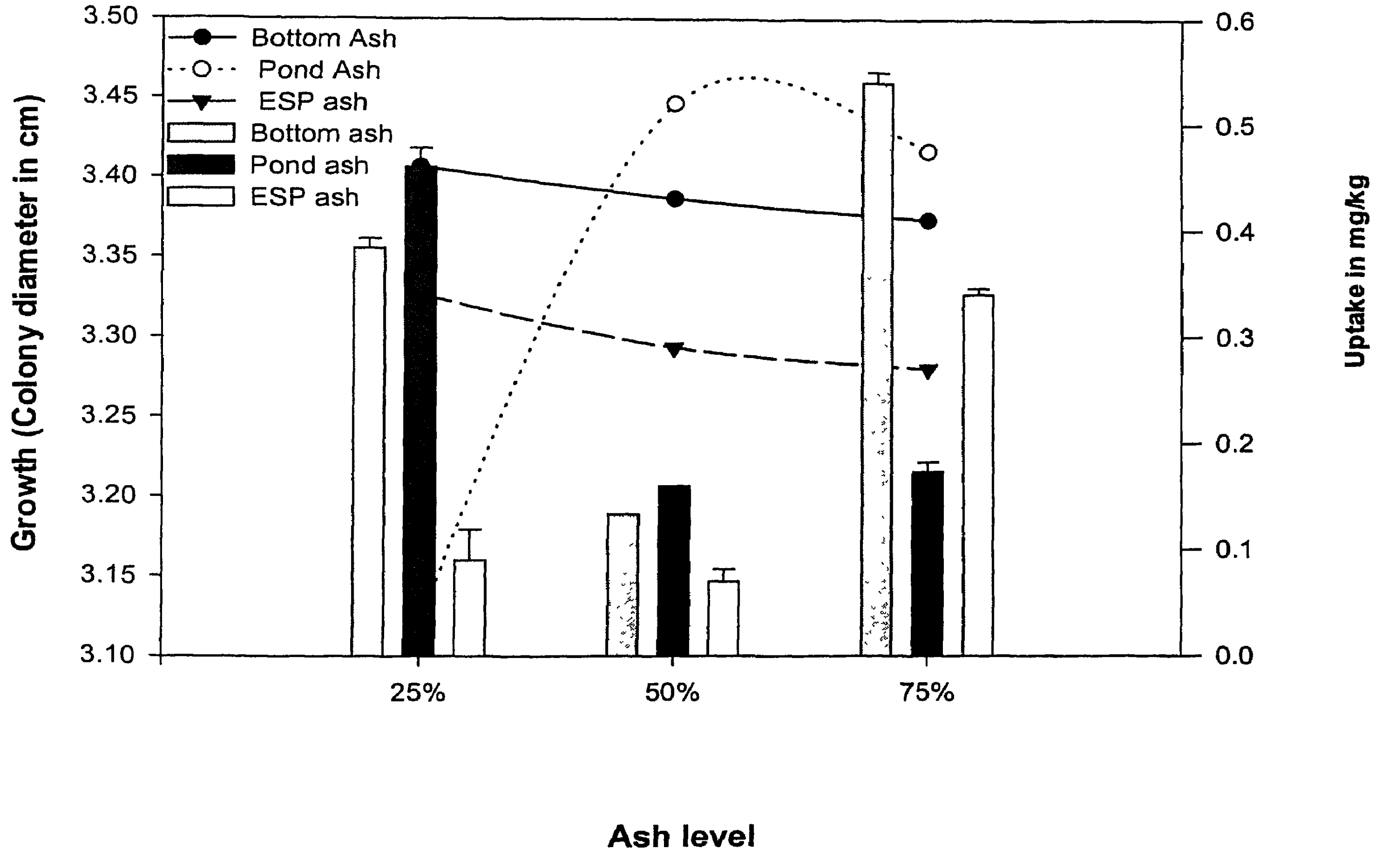


Figure 3.6.6 Growth response and corresponding iron uptake in the mycelia o of *Pisolithus tinctorius* (EM-1293)

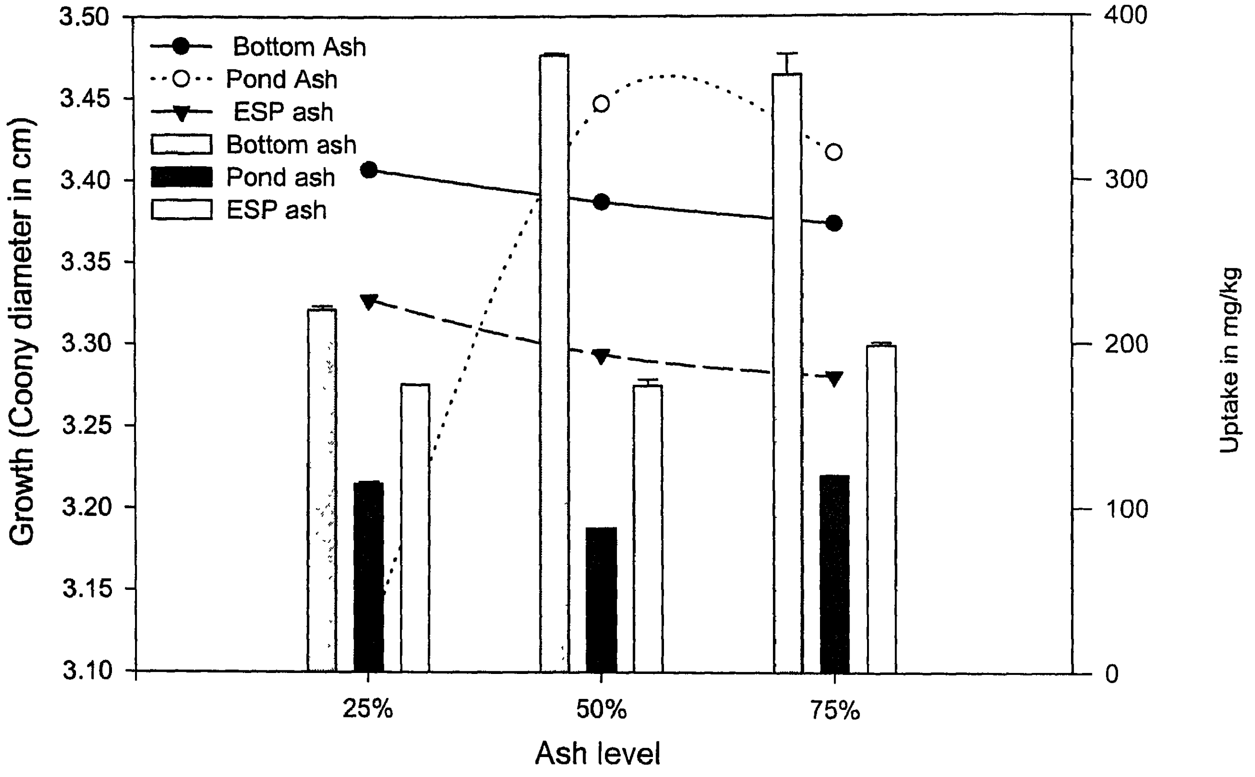


Figure 3.7.1 Growth response and corresponding aluminium uptake in the mycelia of *Scleroderma cepa* (EM-1233)

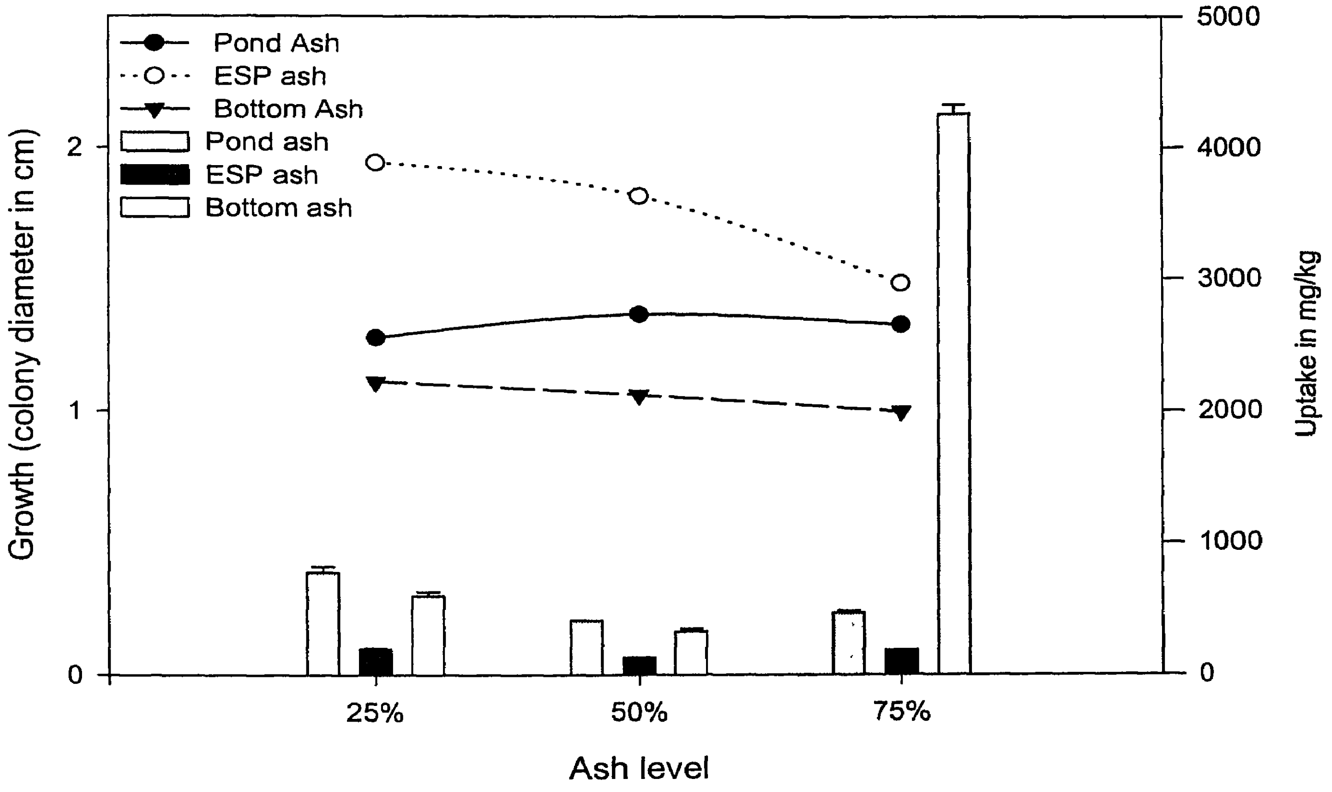


Figure 3.7.2 Growth response and corresponding nickel uptake in the mycelia of *Scleroderma cepa* (EM-1233)

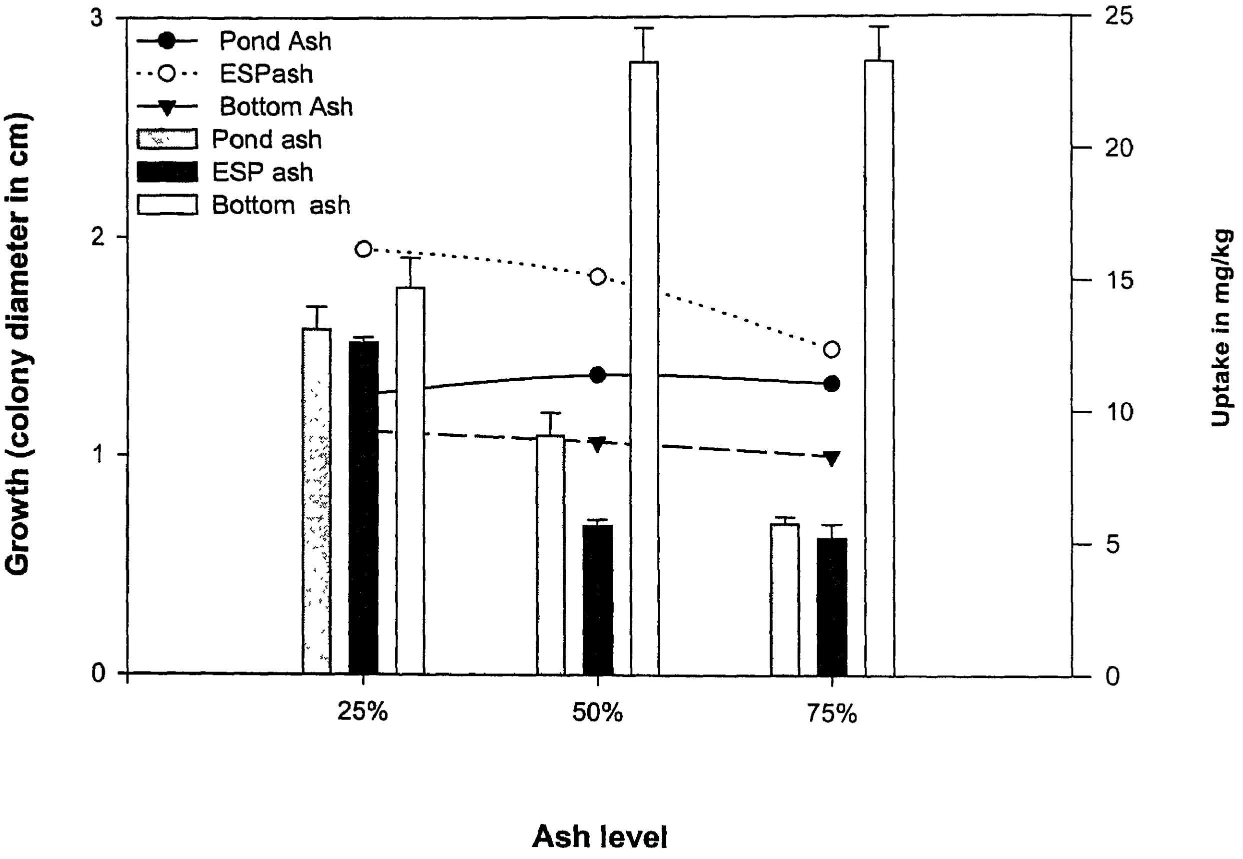


Figure 3.7.3 Growth response and corresponding lead uptake in the mycelia of *Scleroderma cepa* (EM-1233)

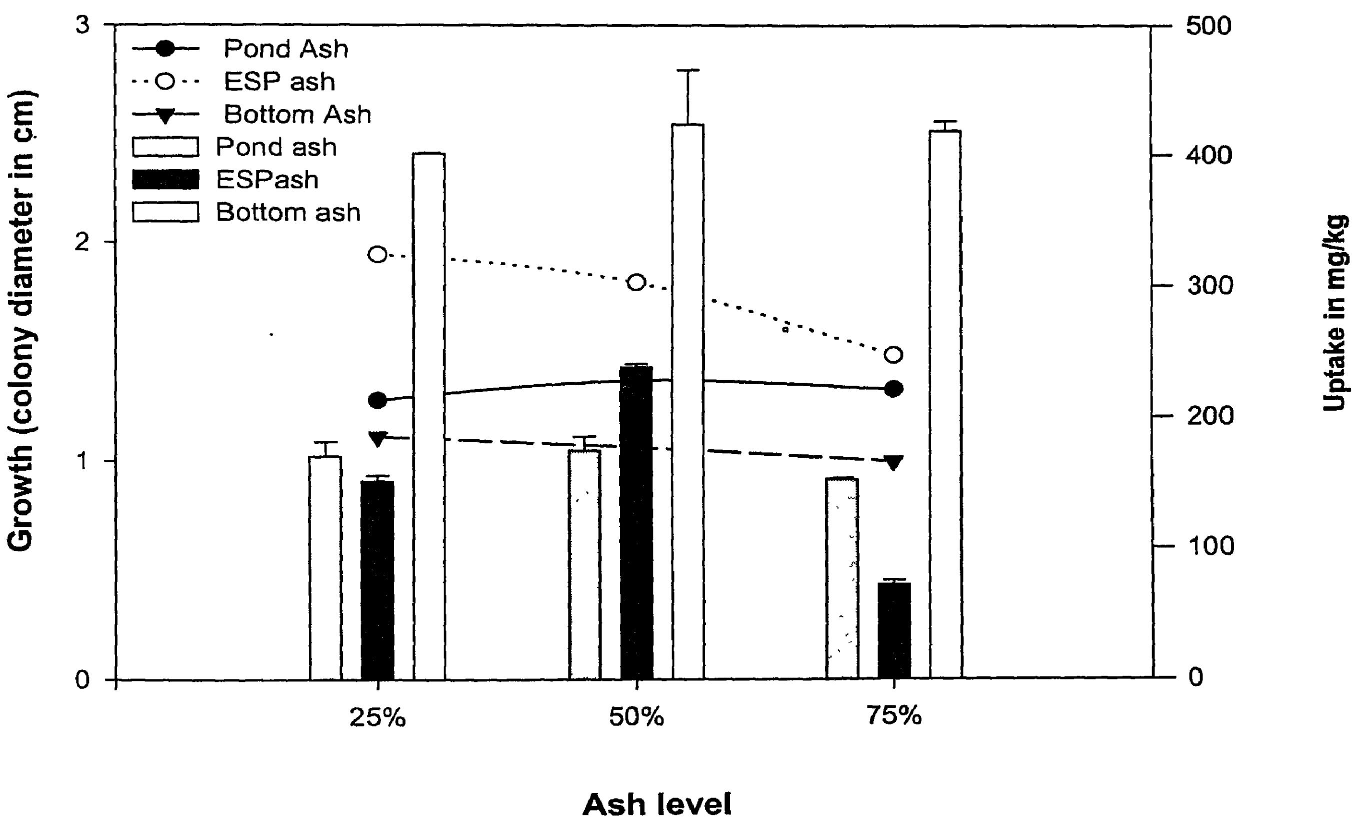


Figure 3.7.4 Growth response and corresponding chromium uptake in the mycelia of *Scleroderma cepa* (EM-1233)

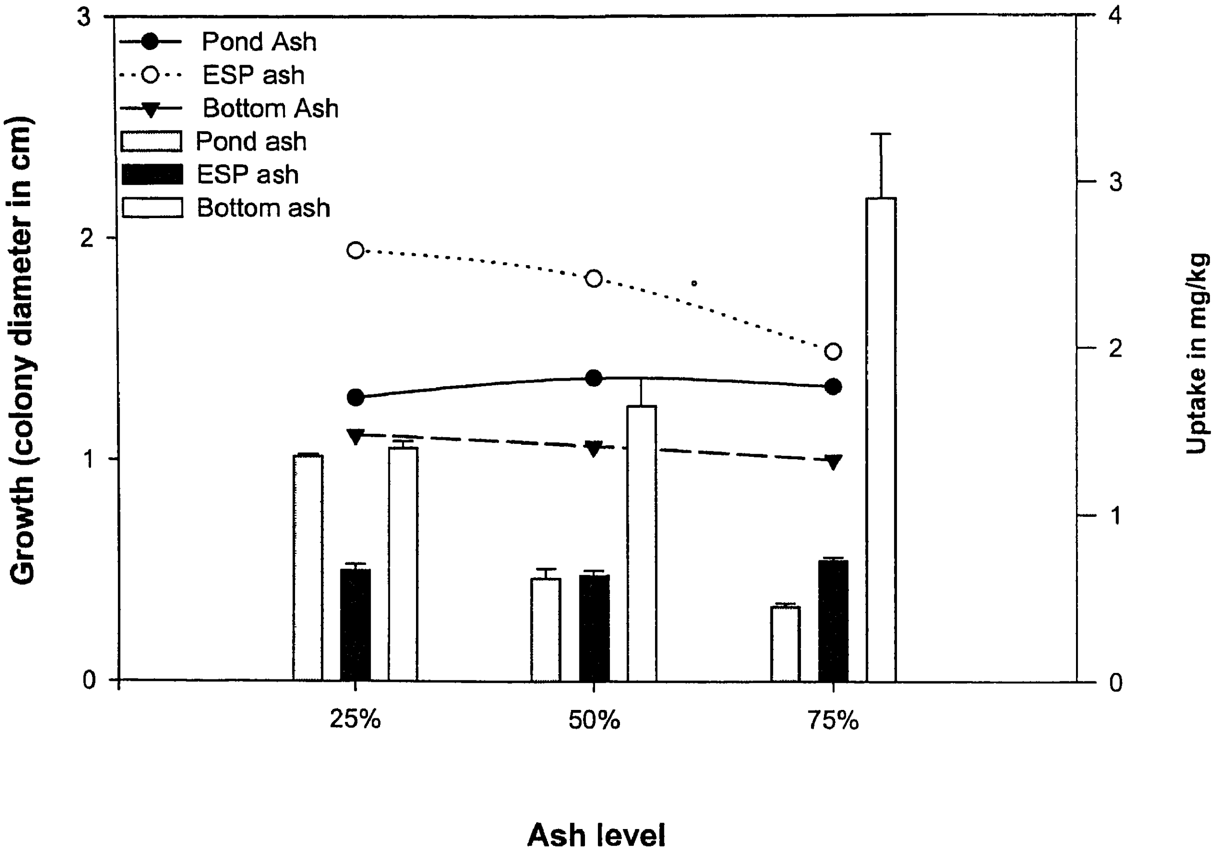


Figure 3.7.5 Growth response and corresponding cadmium uptake in the mycelia of *Scleroderma cepa* (EM-1233)

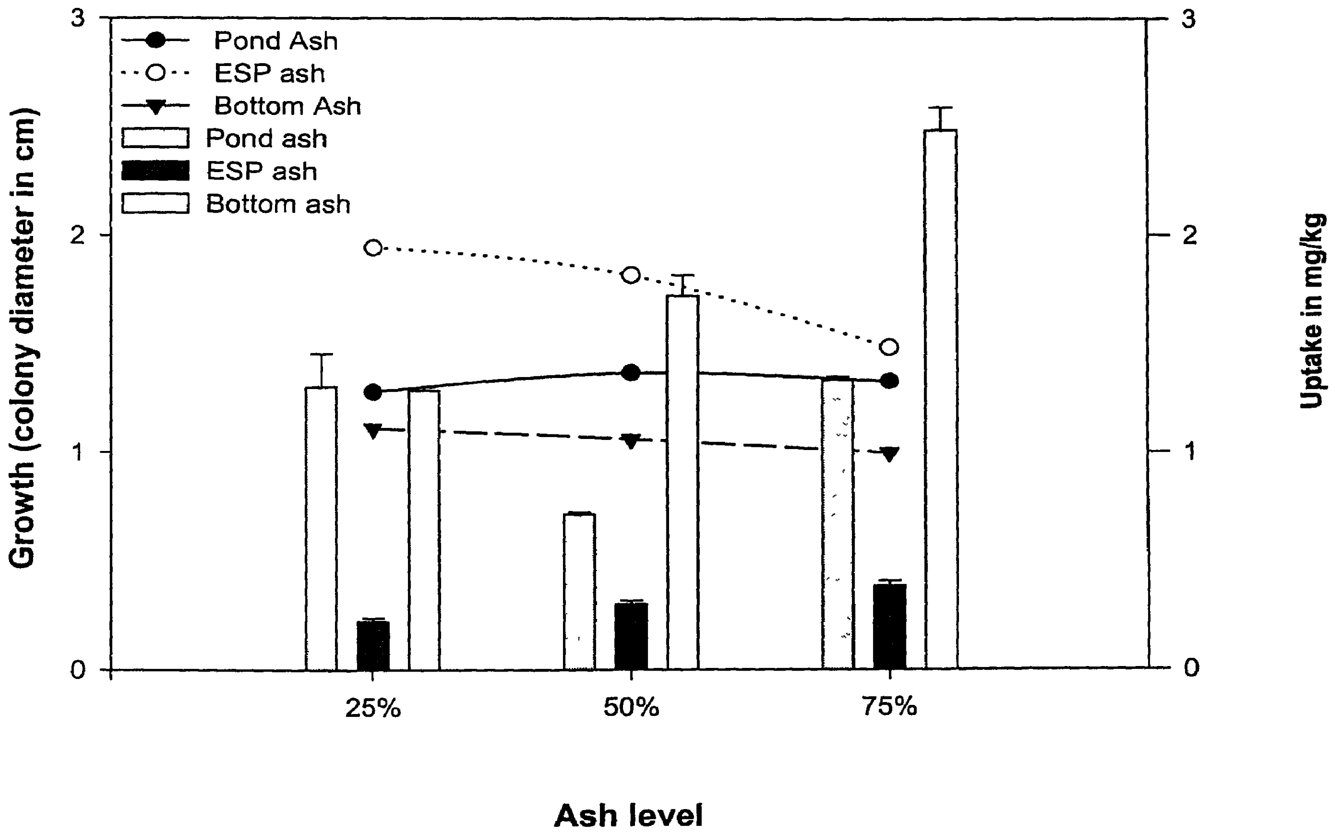


Figure 3.7.6 Growth response and corresponding iron uptake in the mycelia of *Scleroderma cepa* (EM-1233)

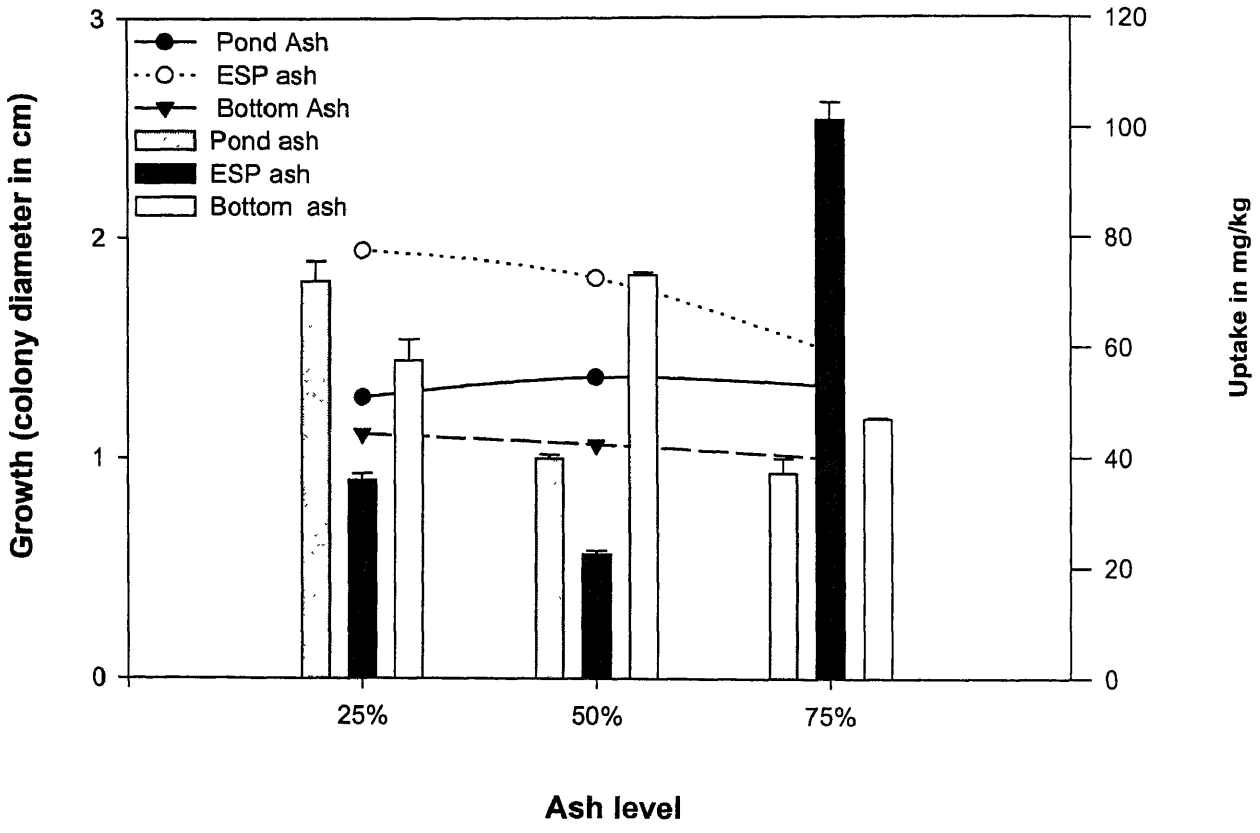


Figure 3.8.1 Growth response and corresponding aluminium uptake in the mycelia of *Scleroderma verucosum* (EM-1283)

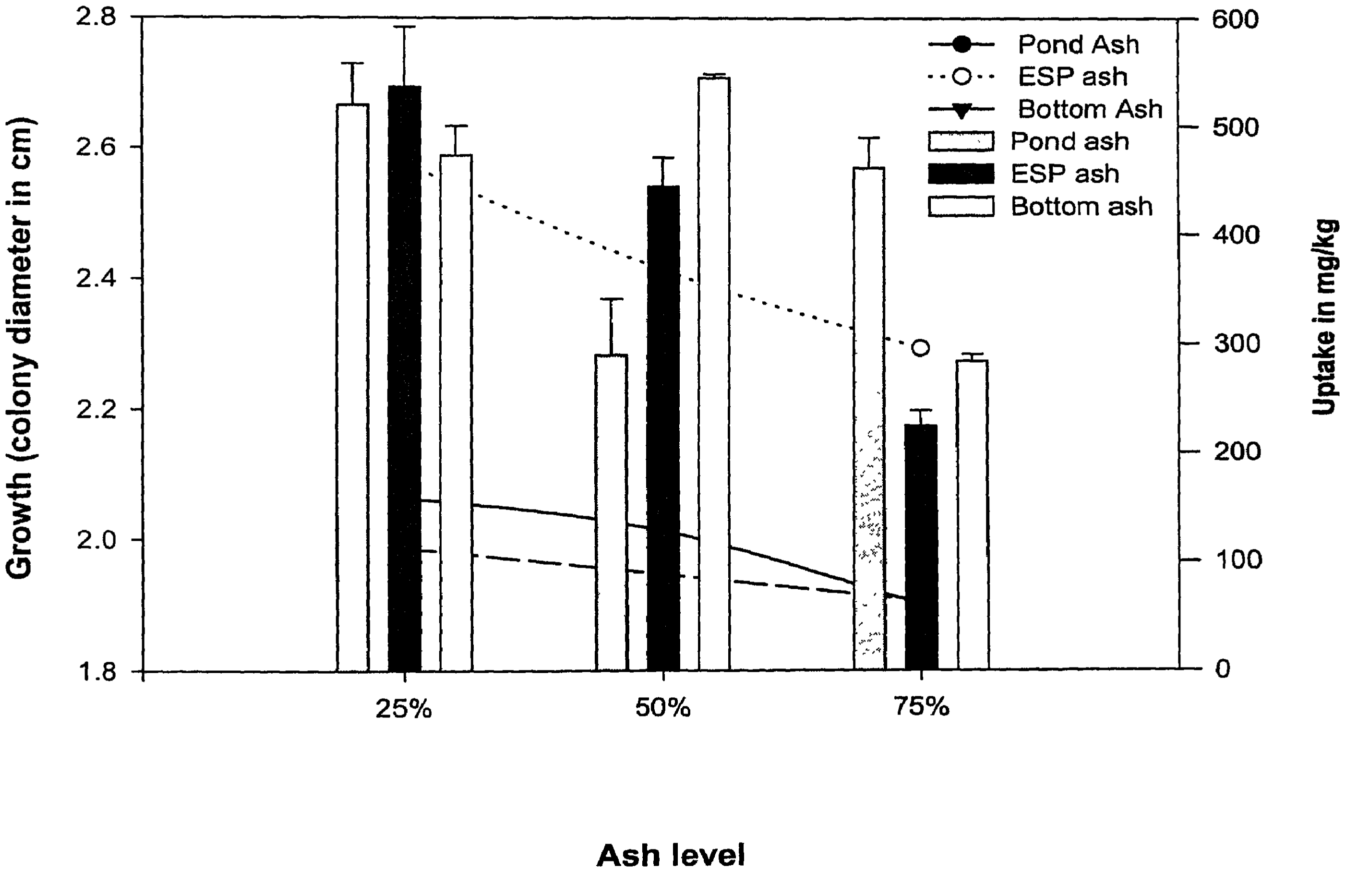


Figure 3.8.2 Growth response and corresponding nickel uptake in the mycelia of *Scleroderma verucosum* (EM-1283)

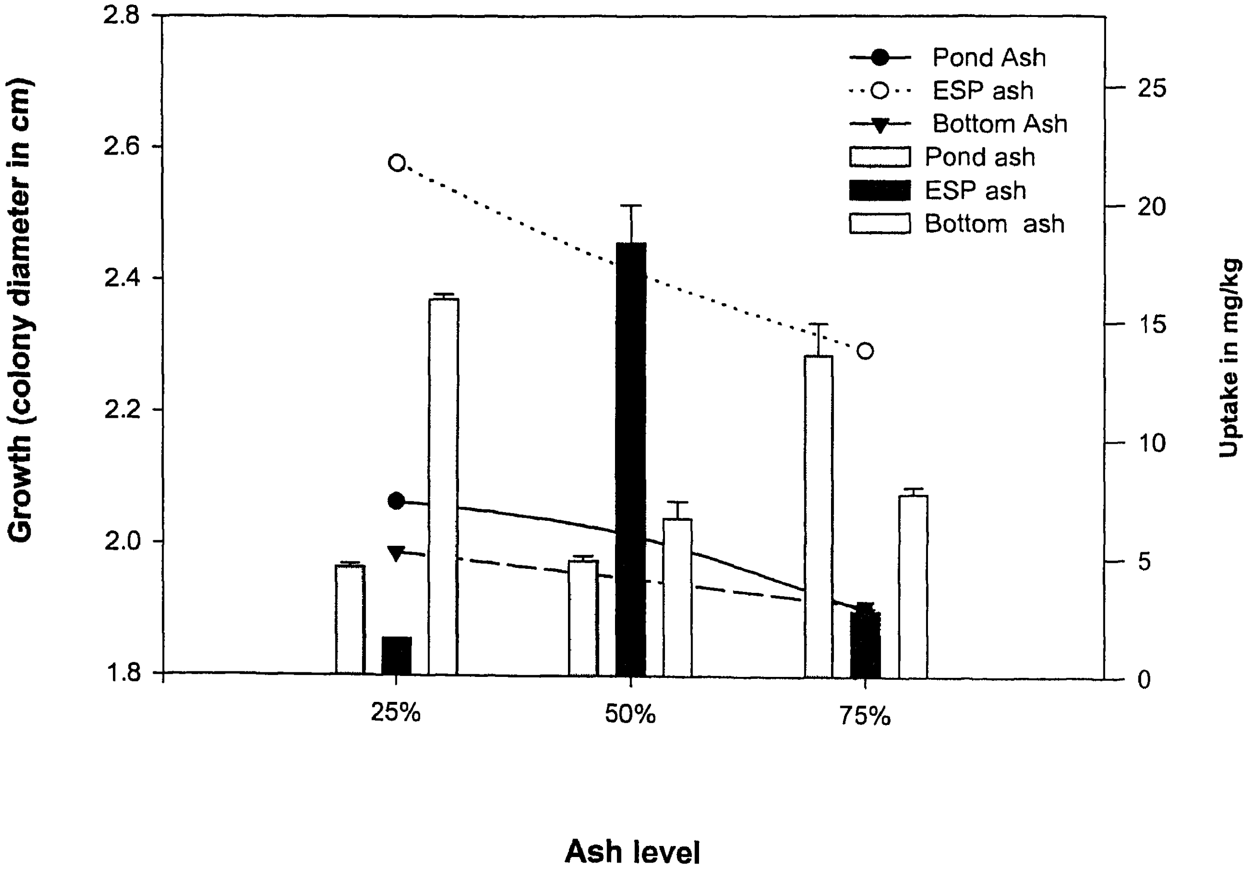


Figure 3.8.3 Growth response and corresponding lead uptake in the mycelia of *Scleroderma verucosum* (EM-1283)

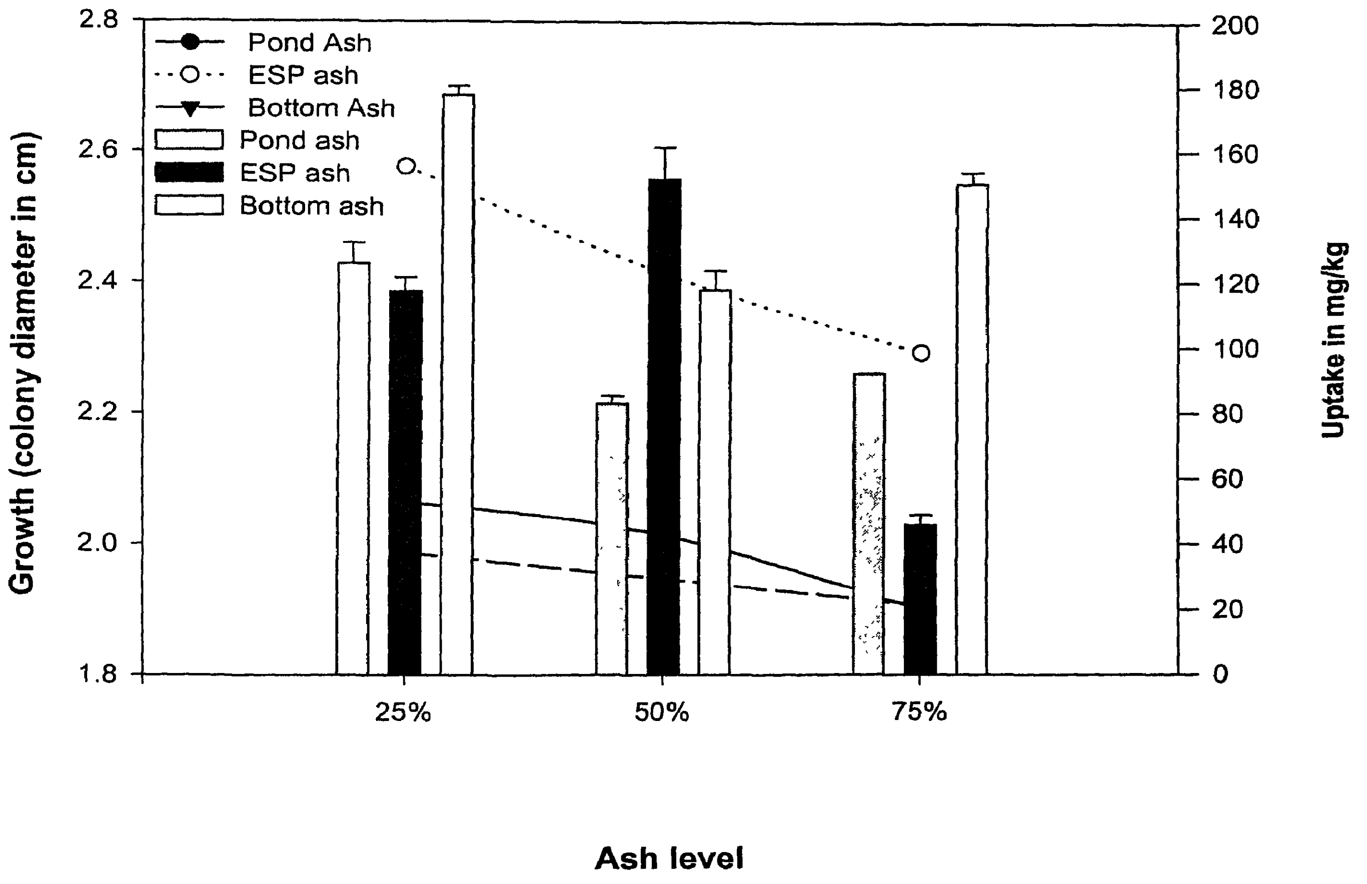


Figure 3.8.4 Growth response and corresponding chromium uptake in the mycelia of *Scleroderma verucosum* (EM-1283)

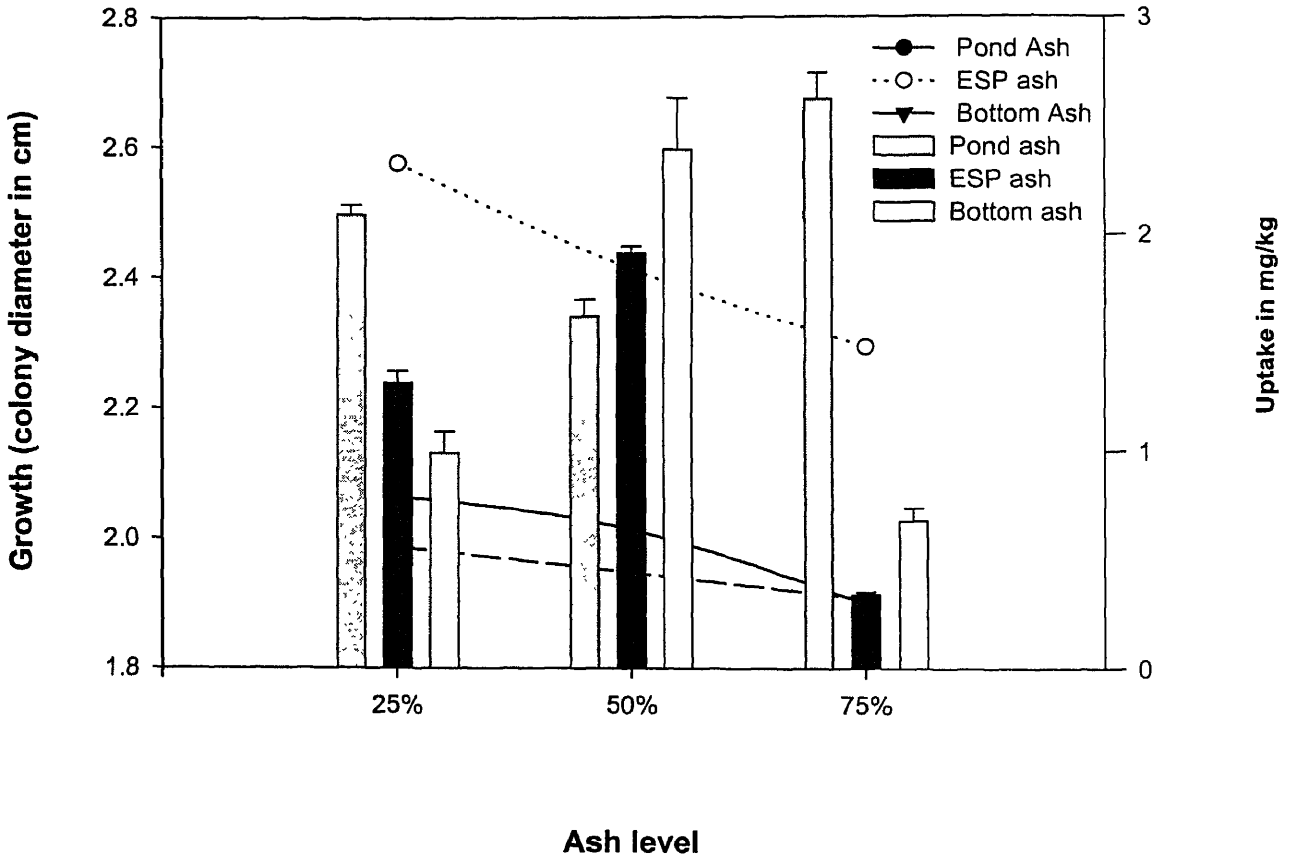


Figure 3.8.5 Growth response and corresponding cadmium uptake in the mycelia of *Scleroderma verucosum* (EM-1283)

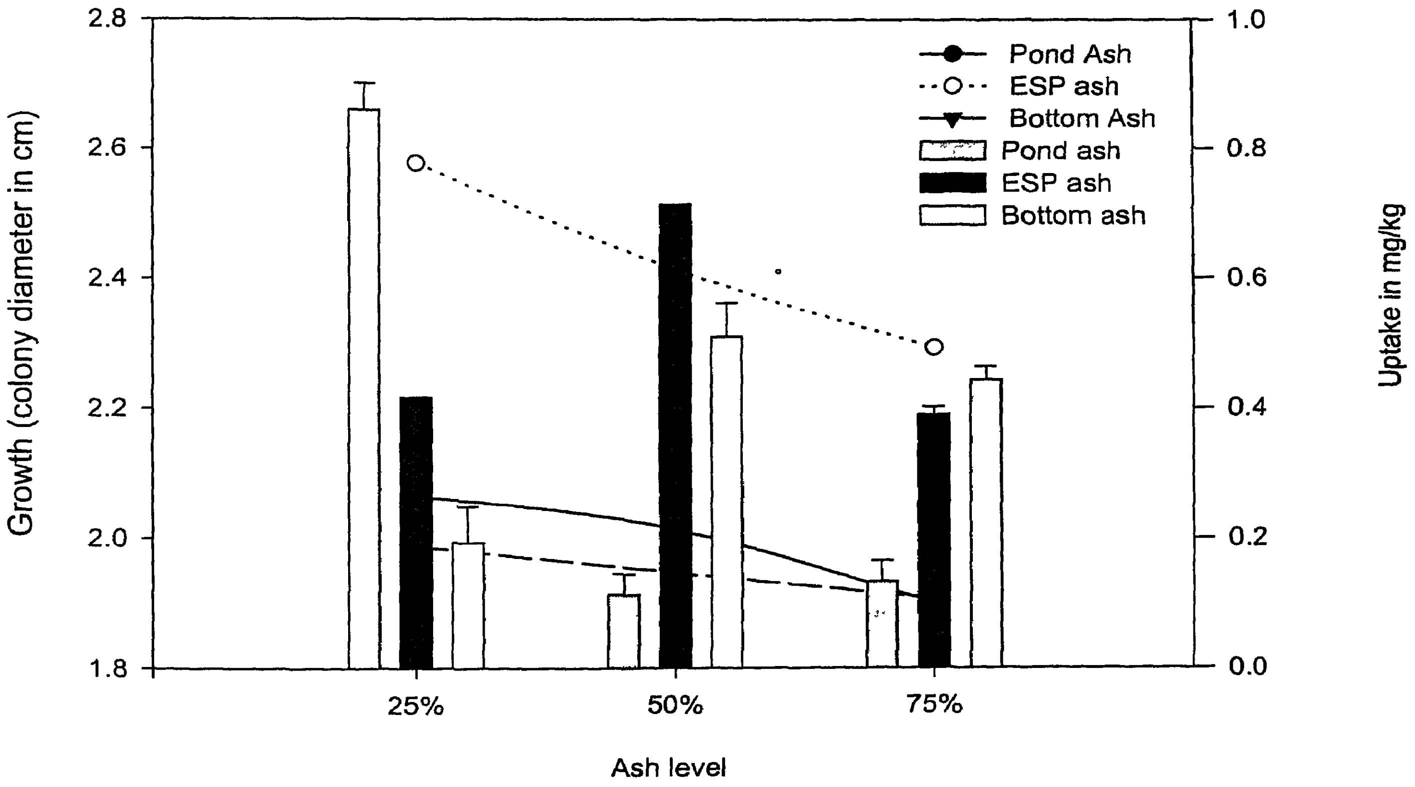
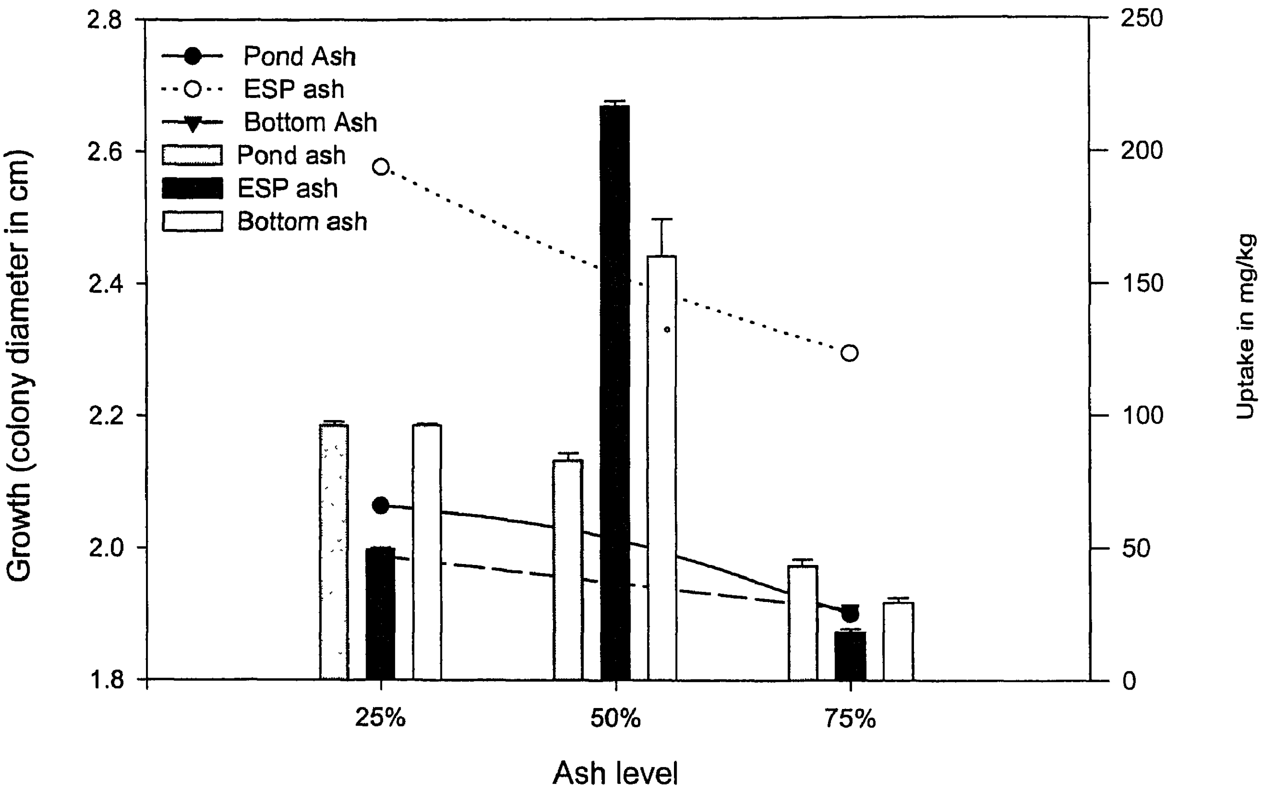


Figure 3.8.6 Growth response and corresponding iron uptake in the mycelia of *Scleroderma verucosum* (EM-1283)



4

Task 3 - Strain collection in nursery conditions

TERI - India

Experiment 1 - Heavy metal uptake by *Sesbania sesban, Mentha arvensis,* and *Vetiver zizanoides* inoculated with VA-mycorrhiza from coal ash.

Introduction

A field experiment was performed to test the effect of vesicular arbuscular mycorrhiza on heavy metal uptake by herbaceous plants. Three herbaceous plants, *Mentha arvensis, Sesbania sesban,* and *Vetiver zizanoides* were grown on coal ash at the Super Thermal Power Station, Korba, Central India. Plants were inoculated with three VA-mycorrhiza: VAM I (mixed consortia from Badarpur, North India); VAM II (*Glomus intraradices* from Badarpur, New Delhi, North India); VAM III (native and mixed consortia from STPS, Korba). Two different levels of farmyard manure treatment were given to the plants. Roots of the plant species were observed for the percentage colonization by VA-mycorrhiza. VA-mycorrhiza II was observed to be better a colonizer than the others irrespective of the hosts tested in this work. Roots and shoots of the plant samples tested were segregated by cutting, followed by grinding of plant tissue. Ground samples were analyzed after digestion for the trace elements and heavy metals, Al, Ni, As, Si, Pb, Cd, and Fe in the roots and the shoots using atomic absorption spectrophotometry. Shoot/root ratio of accumulation of Pb, Ni, Al, Cd, Si, was found to be low in mycorrhiza infected plants. Concentrations of Fe, Al, Ni in roots and shoots was found to be more in plants treated with higher doses of farmyard manure.

Experimental layout at STPS, Korba

The present work was carried out in the TERI' s experimental site at the Super Thermal Power Station, Korba (22°20'N & 82°42'E), Chattisgarh, India. It is situated at a height of 304.8 m above sea level with average rainfall of 1420.08 mm. Here, a 1.43 hectare of land is currently being used for demonstration. The site is filled with 200-ft depth of coal ash. The entire site is divided into three blocks Block A, Block B, and Block C. Block B was chosen for the present study (Figure 4.1).

Tree species planted for reclamation were *Casuarina equisetifolia, Melia azadirach, Albizzia procera,* and *Gmelina arborea.* Herbaceous plant species such as *Mentha arvensis, Vetiver zizanoides, sesbania sesban* were intercropped with the tree species. Three types of mycorrhizal inoculum were introduced. They were designated as VAM I (mixed consortia from the Badarpur site), VAM II (*Glomus intraradices* from the Badarpur site), VAM III (native and mixed consortia from STPS, Korba). Two levels of farmyard manure (FYM II and I) were used. Different dose levels were used for tree species and herbaceous species. For the tree species the doses of FYM were 5 kg/tree and 10 kg/tree (FYM I and II) respectively. For herbaceous species doses of FYM applied were 100 tonnes/ha and 150 tonnes/ha (FYM I and II) respectively.

Table 4.2 Physical characteristics of coal ash

Parameters	*Zero Time*
Bulk density (g/cc)	0.757
Particle density (g/cc)	1.80
Porosity (%)	59.0
Water holding capacity (%)	40.99
Permeability (cm/sec)	6.85 x 10^{-5}
Particle size distribution	
Sand: Medium (%)	2.7
Coarse (%)	0.4
Fine (%)	40.1
Silt (%)	53.1
Clay (%)	3.7

Table 4.3 Chemical characteristics of coal ash

Parameters	*Fly ash 0-15 cm*	*Fly ash 15-30 cm*	*Fly ash 30-90 cm*
pH (1:2.5)	8.77 ± 0.531 (0.237)*	7.97 ± 0.201 (0.090)	7.68 ± 0.132 (0.059)
Cation Exchange Capacity(me/100g)	0.87	0.87	0.87
Electrical conductivity (dS/m)	0.039 ± 0.015 (0.006)	0.035 ± 0.010 (0.004)	0.032 ± 0.003 (0.001)
Nitrogen (total %)	0.10 ± 0.045 (0.020)	0.52 ± 0.017 (0.007)	0.03 ± 0.009 (0.004)
Organic carbon (%)	0.29 ± 0.109 (0.048)	0.21 ± 0.142 (0.063)	0.23 ± 0.076 (0.034)
Potassium (ppm)	77.07 ± 8.24 (5.44)	79.60 ± 6.33 (3.38)	79.36 ± 2.70 (1.20)
Available phosphorus (ppm)	7.08 ± 1.28 (0.575)	7.75 ± 1.59 (0.714)	8.57 ± 1.10 (0.439)
Total Microbial count (cfu)	–	0.2 x 10^3	–
Dehydrogenases activity (μg/g/24 hrs)	–	3.49	–

± Standard deviation; * Standard error of mean

Table 4.4 Level of heavy metals in coal ash

Heavy metals (ppm)	*Zero time*
Chromium	16.42 ± 2.72 (1.57)*
Nickel	57.27 ± 4.44 (2.56)
Cobalt	31.86 ± 3.54 (2.04)
Lead	76.52 ± 3.88 (2.24)
Cadmium	7.95 ± 1.20 (0.69)
Iron (available)	1.64 ± 0.072 (0.04)
Copper (total)	92.03 ± 18.89 (6.49)
Copper (available)	0.12 ± 0.05 (0.03)
Zinc (total)	78.88 ± 9.25 (5.34)
Zinc (available)	2.37 ± 0.87 (0.50)
Manganese (total)	64.99 ± 16.28 (3.14)
Manganese (available)	0.20 ± 0.05 (0.03)
Arsenic	23.48± 6.60 (2.08)

Materials and Methods

Collection of samples

Three herbaceous plant species *Mentha arvensis, Vetiver zizanoides, and Sesbania sesban* were collected from the experimental site at STPS, Korba Chattisgarh. Rhizospheric soil of the plant samples was also collected. Five plants were collected for each sample. Plants and rhizospheric ash samples were separated and kept in paper bags. Altogether 288 samples were collected which finally pooled down to 72 samples. All the samples were brought to the laboratory and maintained in a cold room (temp 4°C) until further processing.

Clearing, staining and quantification of mycorrhizal colonization percentage in roots

Root specimens were taken in a test tube and treated as per the method of Phillips and Hayman (1970), and quantified for mycorrhizal colonization percentage as per Biermann and Lindermann's method (1981).

Sample preparation for heavy metal assay

Adhered ash particles were removed physically and clean plant samples were dried in an oven at 80°C overnight. After drying, the dry weight of the sample was measured. The dried plant samples were separated into root and shoot portions and ground into a fine powder using a mechanical grinder. A small quantity (0.2 g) of powdered shoot, root and subsequent rhizospheric ash were taken and analyzed for heavy metals.

Heavy metal assay using atomic absorption spectophotometry

Root, shoot and ash samples were digested with 5 ml of HNO_3 and 1 ml of HF in a closed vessel at 170°C using MARS5 (Microwave accelerated reaction system 5), CEM Corp for heavy metal analysis. The analysis was carried out by a TJA Solutions (Unicam) AAS (Model *SOLAAR* M Series) system equipped with a GF 95 graphite furnace with FS 95 auto sampler. The data was handled by SOLAAR M Data station (version 8.12) having software version 1.14.

Results

Assessment of percentage colonization

Mycorrhizal colonization results were subjected to both one-way and two-ways *ANOVA* (Table 4.5a and 4.5b) to test for the significant differences caused by FYM and VAM independently and collectively.

Sesbania sesban

The result showed a significant difference in root colonization of *Sesbania* sp. with respect to different VA-mycorrhiza at two different FYM levels. VAM I (mixed consortia from Badarpur site) showed the best colonization ability of all the three, on roots of *Sesbania* irrespective of the FYM dose. Both FYM dose and VA-mycorrhiza had a significant impact on the colonization ability, independently. However interaction of the two did not show any significant change.

Mentha arvensis

VA-mycorrhiza II (*Glomus intraradices*) and VA-mycorrhiza III (native and mixed consortia from Korba site) showed better colonization ability on the roots of *Mentha* sp. Although difference in colonization ability was noted because different inocula were used, no significant difference was noted due to difference in FYM levels.

Vetiver zizanoides

Colonization on vetiver roots was more or less similar by all the types of VA-mycorrhiza. No significant difference in colonization was noted either due to the effect of FYM.

Assessment of heavy metal accumulation

The dried, powdered root, shoot and the rhizospheric soils of the three herbaceous plant species were analyzed for heavy metals to under stand the accumulation of various heavy metals on the root and shoot portions.

Sesbania sesban – heavy metal accumulation profile in the root

The heavy metal accumulation profile of the root of *Sesbania sesban* is reported in Tables 4.6 and 4.6a. Maximum iron uptake was noted in the control i.e. plants without any mycorrhizal treatment. Very poor iron uptake was noticed in the roots infected with VA-mycorrhiza I and II. This could be an indication of immobilization of iron within the mycorrhizal system and prevention of translocation into the root.

Poor accumulation of lead was noticed in roots infected with VA-mycorrhiza I and III at FYM dose II. No difference was noticed in plants with FYM dose I. ANOVA of lead uptake clearly showed the significant impact of FYM levels on it. No significant difference in the nickel uptake was noticed in the roots of *Sesbania sesban*. Poor nickel accumulation was observed in roots treated with VAM III at FYM level I. Aluminum accumulation was least in the roots infected with VA-mycorrhiza I at the FYM level I. Here also a significant impact of FYM dose was noticed on aluminum accumulation. No significant difference in the chromium uptake was noticed. However, accumulation was fairly high in the plants treated with FYM I. Arsenic accumulation was least in the control, and cadmium accumulation was poorest in plants inoculated with VA-mycorrhiza III. Silicon uptake was fairly low in VA-mycorrhiza III infected roots at FYM level II. No significant difference in the arsenic and silicon uptake was noticed due to different mycorrhizal treatments.

Heavy metal accumulation profile in the shoot

The heavy metal accumulation profile in the shoot is reported in Tables 4.7 and 4.7 a. Poor uptake of iron, aluminum, and arsenic, was noticed in the VA-mycorrhiza I inoculated plants. The result showed the significant impact of both, inoculum and FYM level on the accumulation of all metals except aluminum. Less lead and chromium accumulation was observed in plants inoculated with plants infected with VA-mycorrhiza II. Less cadmium accumulation was observed in plants inoculated with plants infected with VA-mycorrhiza II and III. Although little nickel accumulated in the shoots of plants inoculated with

VA-mycorrhiza III and FYM level II there was no significant difference in accumulation of plants treated with FYM level I.

Heavy metal profile of the rhizospheric ash

The heavy metal profile of the rhizospheric ash is reported in Tables 4.8 and 4.8a. Iron and lead content were highest in the rhizospheric ash of the VA-mycorrhiza I inoculated plants treated with FYM II and least in the control. In case of nickel the minimum concentration was reported in the rhizospheric ash of the VA-mycorrhiza II-inoculated plants treated with FYM I. Aluminum and arsenic profile was highest in the control with FYM dose I and least in the control with FYM dose II. No significant difference in the chromium, cadmium, silicon, profile of rhizospheric ash was noticed.

Mentha arvensis- Heavy metal accumulation profile in the root

The heavy metal accumulation profile in the root is reported in Tables 4.9 and 4.9a. Variation in the iron and lead uptake was observed due to the effect of different types of inoculum treated with FYM dose I but no difference was noted in the subsequent treatments with FYM dose II. Similar results were obtained for aluminum and chromium in the roots where significant differences were observed due to the effect of different types of inocula with FYM dose I but not. However just the reverse was observed with arsenic where no variation was observed with different inocula at the FYM level I but there was a variation at the FYM level II. Accumulation of iron, cadmium, arsenic, and nickel was restricted in plants inoculated with VA-mycorrhiza I and accumulation of chromium and silicon was reported to be fairly low in plants inoculated with VA-mycorrhiza II. Low concentrations of lead and aluminum were reported in roots infected with VA-mycorrhiza III. Barring iron, arsenic, and silicon, there were significant impacts of both VA-mycorrhiza and FYM dose on the uptake of other heavy metals.

Heavy metal accumulation profile in the shoot

The heavy metal accumulation profile in the shoot is reported in Tables 4.10 and 4.10a. Low accumulation of all the heavy metals in the shoot was noticed in plants inoculated with VA-mycorrhiza I. However with iron and nickel although variations were noticed with respect to inocula in the FYM level I, no significant difference was noticed in the similar treatments with FYM level II. With chromium significant differences in uptake were noticed in the FYM level II with respect to various inocula but no variation was observed in the subsequent FYM level II. Except for iron, silicon and nickel, FYM and VA-mycorrhiza had a collective, substantial impact on the accumulation of other heavy metals in the shoot.

Heavy metal profile of the rhizospheric ash

The heavy metal profile of the rhizospheric ash is reported in Tables 4.11 and 4.11a. The content of iron and nickel was fairly high, and that of lead was fairly low in the rhizospheric ash of the plants inoculated with VA-mycorrhiza I. Arsenic, aluminum and cadmium contents were low in the rhizospheric ash of the plants inoculated with VA-mycorrhiza II whereas the concentration of chromium was reported to be low in the rhizosphere of the plants inoculated with VA-mycorrhiza III.

Vetiver zizanoides–heavy metal accumulation profile in the root.

The heavy metal accumulation profile in the root is reported in Tables 4.12 and 4.12a. Iron, nickel, arsenic, cadmium, lead accumulation was low in roots infected with VA-mycorrhiza II. Aluminum, silicon, and chromium uptake was very low in the roots infected with VA-mycorrhiza I. Strong impact on uptake levels was noted due to the effect of VA-mycorrhiza and FYM levels.

Heavy metal accumulation profile in the shoot

The heavy metal accumulation profile in the shoot is reported in Tables 4.13 and 4.13a. Iron, aluminum, silicon, chromium, cadmium, were low in the shoots of the *Vetiver zizanoides* infected with VA-mycorrhiza III. Lead accumulation was low in plants inoculated with VA-mycorrhiza II. Nickel and arsenic accumulation was low in VA-mycorrhiza I infected plants. FYM levels did not show any impact on metal accumulation with respect to lead, nickel, chromium, arsenic, and silicon. But the collective impact of FYM and VA-mycorrhiza treatments were prominent in all the cases of heavy metal uptake.

Heavy metal profile of the rhizospheric ash

The heavy metal profile of the rhizospheric ash is reported in Tables 4.14 and 4.14a. No signification difference was noted in the iron concentrations in the rhizospheric ash of the plant with varied VA-mycorrhiza treatments. The nickel profile was similar where no significant difference was noted in FYM level I with respect to inocula but in FYM II. The aluminum concentration was fairly high in VA-mycorrhiza I treated rhizospheric ash. Chromium, cadmium, arsenic and silicon concentrations were fairly high in VA-mycorrhiza III treated rhizospheric ash. FYM level had a strong impact on the heavy metal profile of the rhizospheric soil except for iron, arsenic, and silicon and the dual impact of both FYM and VA-mycorrhiza treatments were significant in all the cases.

Discussion

Among the rhizospheric organisms involved in plant interactions with the soil, arbuscular mycorrhizal fungi deserve special attention. Unfortunately, the role that Glomalean fungi play in plant interactions with soil metals is not fully understood and little is known about mycorrhiza functioning under conditions imposed by metal remediation protocols and about the effects of the phytoextraction on AM fungi. Recently plants capable of forming an association with AM fungi, including maize have been shown to accumulate a considerable amount of metal. Plants reveal various tendencies in the uptake of trace elements. Three general uptake characteristics can be distinguished. They are accumulation, indication, and exclusion. In our present work, poor iron uptake was noticed in the root and shoot of *Sesbania* treated with VA-mycorrhiza. Similar results were also observed in *Vetiver* root and shoot. Not much information is available on the effect of AM fungi in iron uptake by plants. The long-term effect of mycorrhizal colonization Fe concentration the plant tissue and the total plant uptake is variable and the reported results are inconsistent. However effect of AM fungi on plant Fe acquisition strongly depends on experimental conditions such as type of fungus, soil pH. However, differences in total iron uptake or tissue iron concentration between mycorrhizal and non mycorrhizal plants are not direct evidence for the ability of the mycorrhizal fungi to take up iron and deliver it to plants, because other differences between mycorrhizal and non mycorrhizal plants like root physiology may effect iron uptake.

Although enhanced absorption of trace elements from deficient or non enriched soils by AM plants has been documented in the reported literature relatively little attention has been paid to the role of AM fungi in metal-contaminated soils. Under such conditions killham and Firestone found an adverse effect of AM plants on plant growth. Several workers reported that shoot concentrations of Zn, Cu Pb, Cd decreased with AM colonization at high levels of available metals, where at the lower levels, metal uptake increased compared with non-mycorrhizal plants. We observed less Pb and Cd uptake in *Sesbania* inoculated with VA-mycorrhiza II. Similar results were also observed in *Vetiver* shoot where low cadmium accumulation was reported in VA-mycorrhiza III inoculated plants and that of lead in VA-mycorrhiza II inoculated plants. Such reduced transfer as indicated by enhanced root/shoot Cd ratios in AM plants, has been suggested as a barrier in metal transport. This may occur due to intracellular precipitation of metallic cations with PO_4. Uptake into hyphae may be influenced by adsorption on hyphal walls, as chitin has an important metal binding capacity.

Our demonstration showed accumulation of Fe, Ni, As, was significantly lower in the roots and shoots of *Mentha* inoculated with VA-mycorrhiza I. Subsequent root colonizing data shows that VA-mycorrhiza II and VA-mycorrhiza III colonized *Mentha* roots better than VA-mycorrhiza I. This indicates that heavy metal uptake has some effect on the infectivity potential of the VA-mycorrhiza fungi. Al, was significantly lower in the roots and shoots of *Sesbania* inoculated with VA-mycorrhiza I, and subsequent root colonizing data showed that VA-mycorrhiza I colonized *Sesbania* roots better than the others in all the cases irrespective of the FYM dose.

The mechanism underlying reduced metal uptake in AM plants is largely unknown. However, observed trends of restricted metal accumulation in root and shoot certain cases give clues. Selective fungal transport that impairs nonessential elements may be one of such mechanisms. Glomalean hyphae may contribute directly to the uptake and translocation of metals to the host roots, including the micro-nutrients such as Cu and Zn and as well as toxic elements. AM fungi may also sequester toxic metals thereby reducing their availability to the plants. Such metal sequestration may alter the metal translocation pattern in plants leading to metal accumulation in rhizospheric roots and reduced metal transfer to the above ground biomass. Besides evidence for heavy metal binding by sulfhydral compounds has also been shown in AMF fungi. Element localization using technique of electron energy loss spectroscopy of mycorrhizal roots of *Pteridium aquilinum* collected from Cd treated plots showed that most of the Cd in the fungal cytoplasm was located in the polyphosphate granules containing S and N together with Al, Fe, Ti and B. The high concentrations of N and S together with Cd indicates the existence of metallothionein-like peptides in the arbuscular mycorrhizal fungi studied. More indirect effects of AM fungi on rhizosphere characteristics include, changes in pH, microbial communities and root exudation patterns.

Conclusion

With respect to host dependency, VA-mycorrhiza II is a more efficient isolate than the other two. In terms of metal immobilization ability, VA-mycorrhiza I was reported to be a good immobilizer of Fe and Al; VA-mycorrhiza II preferentially arrest Pb accumulation, and VA-mycorrhiza III immobilizes Cd, Cr, and Si from accumulating in plant parts.

Collaboration

Training of Ph.D. student in French laboratory and discussions on results obtained for this task brought out a joint preparation of publication.

One way ANOVA tables

Table No 4.6 a Heavy metal accumulation profile in root of *Sesbania sesban*

Treatment	*Concentration (mg/kg)*							
	Fe		Pb		Ni		Al	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM
VAM I	7171.26 b	6664.26 c	55.52 a	17.52 c	29.26 a	24.55 a	5415.82 d	10070.0
VAM II	6287.59 c	8306.58 a	28.09 b	45.76 a	40.46 a	25.79 a	6239.52 c	10781.9
VAM III	7672.21 b	7470.65 b	25.13 b	19.26 c	18.98 a	23.36 a	7099.09 b	8791.7
Control	10273.66 a	8502.56 a	28.66 b	26.23 b	32.55 a	43.85 a	9861.23 a	8425.7
LSD(0.05)	555.05	434.20	3.43	4.19	38.01	43.15	311.05	1094.
	Cr		As		Cd		Si	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM
VAM I	171.39 a	27.15 a	14.53 a	19.83 a	8.80 a	4.54 b	57624.51 a	44290.3
VAM II	170.63 a	27.11 a	13.99 a	20.08 a	5.52 b	5.75 a	47559.84 a	59326.7
VAM III	165.37 a	24.49 b	10.54 b	11.88 a	2.47 c	3.26 c	62069.26 a	23601.7
Control	85.87 b	25.36 ab	8.19 c	10.56 a	2.33 c	2.74 d	68413.59 a	56772.7
LSD(0.05)	171.38	1.81	0.91	587.57	0.72	0.34	45917.49	728.

Table No 4.7a Heavy metal accumulation profile in the shoot of *Sesbania sesban*

Treatment	*Concentration (mg/kg)*							
	Fe		Pb		Ni		Al	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM
VAM I	2191.48 c	4814.66 b	16.06 b	20.46 b	25.96 a	24.82 a	4001.57 c	11403.51
VAM II	4486.21 b	4484.16 b	11.12 c	24.25 b	26.87 a	25.84 a	6857.99 b	9418.02
VAM III	4597.54 b	6452.98 a	19.05 b	53.25 a	24.09 a	15.95 c	10424.81 a	12256.37
Control	6452.87 a	6465.83 a	37.93 a	21.15 b	25.01 a	20.45 b	10290.1 a	25508.84
LSD(0.05)	284.22	491.19	4.85	4.78	3.26	1.53	527.46	33431.5
	Cr		As		Cd		Si	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM
VAM I	75.09 a	52.53 a	21.48 a	5.68 c	11.69 a	2.33 c	20951.79 d	29381.7
VAM II	29.92 c	36.40 b	23.85 a	5.84 c	7.30 c	2.43 c	22523.14 c	65564.87
VAM III	49.50 b	37.34 b	11.66 b	6.83 b	5.69 d	4.83 b	99518.64 a	9769.14
Control	52.55 b	35.22 b	5.45 c	9.80 a	8.32 b	7.55 a	55723.81 b	6353.09
LSD(0.05)	5.83	3.44	2.98	0.53	0.64	0.58	451.92	555.6

Table No 4.8 a Heavy metal profile of the rhizospheric ash of *Sesbania sesban*

Treatment	*Concentration (mg/kg)*							
	Fe		*Pb*		*Ni*		*Al*	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	18370.55 a	15488.81 b	35.96 a	16.23 b	27.27 c	39.32 b	28850.52 c	34757.45 a
VAM II	14712.49 d	11408.48 a	29.03 b	23.90 a	18.49 d	47.16 a	22397.13 d	32708.23 b
VAM III	15300.71 c	14470.61 c	20.88 c	22.89 a	47.18 a	39.66 b	33826.46 b	22476.02 c
Control	16154.02 b	10833.78 d	15.93 d	23.56 a	41.06 b	32.61 c	35011.09 a	9304.564 d
LSD(0.05)	573.28	610.85	0.98	2.56	3.21	3.58	680.95	495.93
	Cr		*As*		*Cd*		*Si*	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	102.50 ab	96.34 a	44.95 b	65.32 a	15.96 b	41.14 a	185742.9 c	190639.2a
VAM II	104.52 a	96.09 a	33.36 d	30.41 b	39.06 a	9.54 b	205176.1 a	188225.2 a
VAM III	108.66 a	87.59 b	38.05 c	34.54 b	6.36 c	5.29 b	189178.3 b	203469.1 a
Control	96.40 b	99.39 a	86.22 a	9.12 c	7.58 c	5.22 b	162229.2 d	131058.9 a
LSD(0.05)	6.83	5.21	3.98	9.81	1.94	19.28	195.50	97213.38

Table No 4.9 a Heavy metal accumulation profile in the root of *Mentha arvensis*

Treatment	*Concentration (mg/kg)*							
	Fe		Pb		Ni		Al	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	5116.46 d	6808.88 a	35.78 a	19.22 a	15.75 c	33.39 ab	11198.88 b	3714.04 b
VAM II	8319.11 a	5536.78 a	21.86 b	50.59 a	21.92 b	34.98 a	16538.09 a	3167.53 b
VAM III	7558.28 b	7523.63 a	8.76 d	29.96 a	25.51 a	35.30 a	6467.48 c	2260.58 b
Control	6360.34 c	8074.89 a	17.01 c	53.07 a	26.16 a	26.68 b	5062.97 d	1929.10 a
LSD(0.05)	331.89	262.56	4.47	38.70	3.24	7.26	494.93	70.48
	Cr		As		Cd		Si	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	422.47 b	367.17 b	11.89 a	6.73 c	2.28 c	7.55 a	54527.09 a	31385.56 c
VAM II	388.85 c	403.46 b	7.82 a	14.01 b	3.30 b	5.39 b	40444.46 b	13291.08 d
VAM III	440.67 a	368.11 b	49.30 a	12.79 b	13.29 a	4.66 c	28332.17 c	66095.31 a
Control	226.92 d	651.66 a	12.11 a	16.09 a	3.50 b	2.42 d	16719.92 d	39479.64 b
LSD(0.05)	11.30	53.57	65.12	1.22	0.48	0.73	723.91	730.82

Table No 4.10 a Heavy metal accumulation profile in the shoot of *Mentha arvensis*

Treatment	*Concentration (mg/kg)*							
	Fe		Pb		Ni		Al	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	6388.40 c	7690.44 a	17.87 b	10.88 c	18.49 b	22.49 a	6815.10 d	10594.79 c
VAM II	8180.06 bc	10675.96 a	36.39 a	16.19 b	23.04 ab	24.44 a	7321.81 c	10564.43 c
VAM III	11633.63 a	11667.84 a	13.64 c	23.45 a	27.00 a	24.43 a	12241.63 b	15516.44 a
Control	10249.77 ab	7739.46 a	8.81 d	17.50 b	25.89 a	25.03 a	13526.82 a	11684.3 b
LSD(0.05)	2513.62	3869.13	4.06	3.26	5.51	5.20	257.46	681.92
	Cr		As		Cd		Si	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	24.72 a	24.99 c	9.49 c	9.86 d	12.32 a	5.81 a	11551.16 b	42406.44 d
VAM II	24.69 a	28.66 b	10.23 b	25.02 a	13.06 a	5.65 a	12259.46 b	53767.09 c
VAM III	24.54 a	23.91 c	10.69 b	12.20 c	9.72 b	6.49 a	67691.45 ab	80545.71 a
Control	18.73 b	44.48 a	12.06 a	15.08 b	8.73 b	6.45 a	66508.32 a	55352.26 b
LSD(0.05)	1.05	2.51	0.67	1.11	1.23	0.80	36772.68	393.43

Table No 4.11a Heavy metal profile of the rhizospheric ash of *Mentha arvensis*

Treatment	*Concentration (mg/kg)*							
	Fe		*Pb*		*Ni*		*Al*	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	22432.24 a	25659.34 a	20.06 b	23.59 b	32.57 a	33.74 a	12603.5 b	14510.05 c
VAMII	18680.05 c	21787.14 b	16.23 c	26.26 b	27.10 b	23.89 c	12514.68 b	13697.98 d
VAM III	16470.18 d	19536.59 c	15.56 c	35.70 a	33.11 a	25.84 b	15547.41 a	28139.28 b
Control	21530.67 b	18401.28 c	23.19 a	33.93 a	27.36 b	19.08 d	15496.05 a	43477.97 a
LSD(0.05)	545.47	1582.38	2.55	3.67	1.88	1.62	539.53	713.45
	Cr		*As*		*Cd*		*Si*	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	85.52 a	81.37 b	20.27 b	36.14 c	12.40 c	25.08 a	103182.5a	148715.1 d
VAM II	88.01 a	85.62 b	16.49 c	33.61 c	15.39 a	13.91 c	160499.1 b	164538.3 c
VAM III	78.04 b	83.09 a	18.88 b	55.29 a	14.34 b	14.32 bc	161488.4 b	168519.1 b
Control	80.28 b	107.46 a	27.52 a	47.34 b	4.21 d	15.29 b	139254.3 b	176639.2 a
LSD(0.05)	3.12	5.12	1.59	3.13	0.66	1.11		527.43

Table No 4.12a Heavy metal accumulation profile in the root of *Vetiver zizanoides*

Treatment	*Concentration(mg/kg)*							
	Fe		Pb		Ni		Al	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	3365.44 b	4673.73 b	191.48 a	87.51 a	32.62 d	45.13 a	1813.59 d	7621.00 c
VAM II	2644.56 c	5741.15 a	79.62 d	73.15 b	35.70 c	18.51 c	2390.40 c	26729.24 d
VAM III	3709.00 a	5536.24 a	108.18 c	82.72 a	40.47 b	43.53 a	6105.20 a	11561.59 a
Control	3768.58 a	5453.18 a	157.55 b	55.48 c	46.80 a	27.99 b	3800.24 b	8674.00 b
LSD(0.05)	295.25	321.65	17.40	5.68	3.02	3.08	128.76	124.48
	Cr		As		Cd		Si	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	148.95 c	627.59 a	12.30 a	11.84 c	13.61 a	13.42 a	1748.98 c	20471.89 d
VAM II	167.25 c	451.12 b	6.30 c	9.72 d	2.14 d	5.01 c	1668.93 c	40082.17 c
VAM III	375.48 b	642.09 a	6.78 c	13.88 b	6.65 b	5.09 c	20005.79 b	69659.38 a
Control	678.05 a	657.19 b	11.14 b	34.35 a	3.25 c	8.61 b	30035.75 a	48340.86 b
LSD(0.05)	20.66	67.64	0.97	1.93	0.77	0.74	891.19	1387.50

Table No 4.13a Heavy metal accumulation profile in the shoot of *Vetiver zizanoides*

Treatment	*Concentration (mg/kg)*							
	Fe		Pb		Ni		Al	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	5190.85 b	2790.41 c	35.13 a	41.97 a	18.06 b	16.30 c	6677.67 a	4899.05 c
VAM II	5098.19 b	5280.86 a	18.67 c	24.90 b	20.21 ab	23.77 a	6355.05 b	5408.06 b
VAM III	4103.67 c	2162.33 d	36.01 a	27.43 b	20.98 ab	19.05 b	4064.39 d	4338.36 d
Control	5639.57 a	3496.88 b	27.74 b	26.65 b	23.96 a	24.20 a	5680.73 c	5683.38 a
LSD(0.05)	309.86	301.15	4.47	3.20	3.66	271.21	181.24	271.21
	Cr		As		Cd		Si	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	28.70 a	25.20 ab	7.03 b	2.51 d	3.38 b	7.72 ab	21609.48 b	23561.9 b
VAM II	26.82 b	24.68 b	8.80 a	4.48 c	3.74 a	7.21 b	2859 5.32 a	42321.27 a
VAM III	22.99 d	25.11 ab	4.23 c	9.62 a	3.34 b	5.38 c	18756.24 c	15547.22 c
Control	24.95 c	26.30 c	7.03 b	7.67 b	2.67 c	8.49 a	14363.09 d	1649.323 ds
LSD(0.05)	1.51	1.25	1.64	1.69	0.34	0.83	556.81	694.03

Table No 4.14a Heavy metal profile of rhizospheric ash of *Vetiver zizanoides*

Treatment	*Concentration (mg/kg)*							
	Fe		*Pb*		*Ni*		*Al*	
FYM dose	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAMI	19759.82 b	20527.10 a	17.17 a	11.02 c	24.59 b	28.84 b	10677.96 a	11168.21 ab
VAM II	18302.08 b	15835.88 a	9.279 b	14.22 b	23.23 b	31.95 a	6306.36 c	9103.46 ab
VAM III	24186.36 a	22973.47 a	11.55 b	14.58 b	22.89 b	32.67 a	8303.78 b	14166.96 a
Control	18833.12 b	16356.41 a	11.83 b	26.97 a	32.17 a	32.29 a	8186.17 b	10439.71 b
LSD(0.05)	3292.66	11838.11	4.67	3.13	2.51	1.52	200.88	6037.72
	Cr		*As*		*Cd*		*Si*	
FYM dose	*FYM I*	*FYM II*	*FYM I*	*FYM II*	*FYM I*	*FYM II*	*FYM I*	*FYM II*
VAMI	43.04 c	50.64 c	20.22 c	20.91 c	3.46 c	4.47 d	138495.40 bc	145792.00 c
VAM II	25.51 d	36.03 d	20.44 c	43.76 a	11.72 a	13.67 b	15 1023.90 b	145942.10 c
VAM III	55.64 b	54.07 b	35.19 b	34.80 b	11.78 a	19.84 a	172 495.60 a	153490.50 b
Control	59.45 a	71.91 a	42.25 a	18.99 d	5.32 b	10.29 c	1284 63.00 c	158978.70 a
LSD(0.05)	2.41	3.37	2.58	1.81	0.56	1.59	14470.33	4001.29

ANOVA tables

Table 4.6 Heavy metal accumulation profile in root of *Sesbania sesban*

Treatment		Concentration (mg/kg)							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	7171.26	55.52	29.26	5415.82	171.39	14.53	8.80	57624.51
	VAM II	6287.59	28.09	40.46	6239.52	170.63	13.99	5.52	47559.84
	VAM III	7672.21	25.13	18.98	7099.09	165.37	10.54	2.47	62069.26
	Control	10273.66	28.66	32.55	9861.23	85.87	8.19	2.33	68413.59
FYM II	VAM I	6664.26	17.52	24.55	10070.01	27.15	19.83	4.54	44290.34
	VAM II	8306.58	45.76	25.79	10781.99	27.11	20.08	5.75	59326.72
	VAM III	7470.65	19.26	23.36	8791.77	24.49	11.88	3.26	23601.70
	Control	8502.56	26.23	43.85	8425.79	25.36	10.56	2.74	56772.75
Main effects									
Inoculum		***	***	ns	***	***	ns	***	ns
FYM		ns	***	ns	***	***	ns	***	ns
Interaction									
FYM X Inoculum		***	***	ns	***	***	ns	***	ns

Table 4.7 Heavy metal accumulation profile in the shoot of *Sesbania sesban*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	2191.48	16.06	25.96	4001.57	75.09	21.48	11.69	20951.79
	VAM II	4486.21	11.12	26.87	6857.99	29.92	23.85	7.30	22523.14
	VAM III	4597.54	19.05	24.09	10424.81	49.50	11.66	5.69	99518.64
	Control	6452.87	37.93	25.01	10290.1	52.55	5.45	8.32	55723.81
FYM II	VAM I	4814.66	20.46	24.82	11403.51	52.53	5.68	2.33	29381.7
	VAM II	4484.16	24.25	25.84	9418.02	36.40	5.84	2.43	65564.87
	VAM III	6452.98	53.25	15.95	12256.37	37.34	6.83	4.83	9769.14
	Control	6465.83	21.15	20.45	25508.84	35.22	9.80	7.55	6353.09
Main effects									
Inoculum		***	***	***	ns	***	***	***	***
FYM		***	***	***	ns	***	***	***	***
Interaction									
FYM X Inoculum		***	***	***	ns	***	***	***	***

Table 4.8 Heavy metal profile of the rhizospheric ash of *Sesbania sesban*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	18370.55	35.96	27.27	28850.52	102.50	44.95	15.96	185742.9
	VAM II	14712.49	29.03	18.49	22397.13	104.52	33.36	39.06	205176.1
	VAM III	15300.71	20.88	47.18	33826.46	108.66	38.05	6.36	189178.3
	Control	16154.02	15.93	41.06	35011.09	96.40	86.22	7.58	162229.2
FYM II	VAM I	15488.81	16.23	39.32	34757.45	99.47	65.32	41.14	190639.2
	VAM II	11408.48	23.90	47.16	32708.23	96.09	30.41	9.54	188225.2
	VAM III	14470.61	22.89	39.66	22476.02	87.59	34.54	5.29	203469.1
	Control	10833.78	23.56	32.61	9304.564	99.39	9.12	5.22	131058.9
Main effects									
Inoculum		***	***	***	***	ns	***	***	ns
FYM		***	***	***	***	***	***	ns	ns
Interaction									
FYM X Inoculum		***	***	***	***	***	***	***	ns

Table 4.9 Heavy metal accumulation profile in the root of *Mentha arvensis*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	5116.46	35.78	15.75	11198.88	422.47	11.89	2.28	54527.09
	VAM II	8319.11	21.86	21.92	16538.09	388.85	7.82	3.30	40444.46
	VAM III	7558.28	8.76	25.51	6467.48	440.67	49.30	13.29	28332.17
	Control	6360.34	17.01	26.16	5062.97	226.92	12.11	3.50	16719.92
FYM II	VAM I	6808.88	19.22	33.39	3714.04	367.17	6.73	7.55	125723.8
	VAM II	5536.78	50.59	34.98	3167.53	403.46	14.01	5.39	52342.57
	VAM III	7523.63	29.96	35.30	2260.58	368.11	12.79	4.66	66095.31
	Control	32453.23	53.07	26.68	1929.10	651.66	16.09	2.42	39479.64
Main effects									
Inoculum		ns	ns	**	***	**	ns	***	ns
FYM		ns	**	***	***	***	ns	**	ns
Interaction									
FYM X Inoculum		ns	*	***	***	***	ns	***	ns

Table 4.10 Heavy metal accumulation profile in the shoot of *Mentha arvensis*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	6388.40	17.87	18.49	6815.10	24.72	9.49	12.32	11551.16
	VAM II	8180.06	36.39	23.04	7321.81	24.69	10.23	13.06	12259.46
	VAM III	11633.63	13.64	27.00	12241.63	24.54	10.69	9.72	67691.45
	Control	10249.77	8.81	25.89	13526.82	18.73	12.06	8.73	66508.32
FYM II	VAM I	7690.44	10.88	22.49	10594.79	24.99	9.86	5.81	42406.44
	VAM II	10675.96	16.19	24.44	10564.43	28.66	25.02	5.65	53767.09
	VAM III	11667.84	23.45	24.43	15516.44	23.91	12.20	6.49	80545.71
	Control	7739.46	17.50	25.03	11684.3	44.48	15.08	6.45	55352.26
Main effects									
Inoculum		**	***	***	***	***	***	***	***
FYM		ns	ns	ns	***	***	***	***	***
Interaction									
FYM X Inoculum		ns	***	ns	***	***	***	***	*

Table 4.11 Heavy metal profile of the rhizospheric ash of *Mentha arvensis*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	22432.24	20.06	32.57	12603.5	85.52	20.27	12.40	103182.5
	VAMII	18680.05	16.23	27.10	12514.68	88.01	16.49	15.39	160499.1
	VAM III	16470.18	15.56	33.11	15547.41	78.04	18.88	14.34	161488.4
	Control	21530.67	23.19	27.36	15496.05	80.28	27.52	4.21	139254.3
FYM II	VAM I	25659.34	23.59	33.74	14510.05	81.37	36.14	25.08	148715.1
	VAM II	21787.14	26.26	23.89	13697.98	85.62	33.61	13.91	164538.3
	VAM III	19536.59	35.70	25.84	28139.28	83.09	55.29	14.32	168519.1
	Control	18401.28	33.93	19.08	43477.97	107.46	47.34	15.29	176639.2
Main effects									
Inoculum		***	***	***	***	***	***	***	ns
FYM		***	***	***	***	***	***	***	*
Interaction									
FYM X Inoculum		***	***	**	***	***	***	***	*

Table 4.12 Heavy metal accumulation profile in the root of *Vetiver zizanoides*

Treatment		Concentration(mg/kg)							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	3365.44	191.48	32.62	1813.59	148.95	12.30	13.61	1748.98
	VAM II	2644.56	79.62	35.70	2390.40	167.25	6.30	2.14	1668.93
	VAM III	3709.00	108.18	40.47	6105.20	375.48	6.78	6.65	20005.79
	Control	3768.58	157.55	46.80	3800.24	678.05	11.14	3.25	30035.75
FYM II	VAM I	4673.73	87.51	45.13	7621.00	627.59	11.84	13.42	20471.89
	VAM II	5741.15	73.15	18.51	26729.24	451.12	9.72	5.01	40082.17
	VAM III	5536.24	82.72	43.53	11561.59	642.09	13.88	5.09	279405.60
	Control	5453.18	55.48	27.99	8674.00	657.19	34.35	8.61	48340.86
Main effects									
Inoculum		***	***	***	ns	***	***	***	***
FYM		***	***	***	ns	***	***	***	***
Interaction									
FYM X Inoculum		***	***	***	ns	***	***	***	***

Table 4.13 Heavy metal accumulation profile in the shoot of *Vetiver zizanoides*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAM I	5190.85	35.13	18.06	6677.67	28.70	7.03	3.38	21609.48
	VAM II	5098.19	18.67	20.21	6355.05	26.82	8.80	3.74	28595.32
	VAM III	4103.67	36.01	20.98	4064.39	22.99	4.23	3.34	18756.24
	Control	5639.57	27.74	23.96	5680.73	24.95	7.03	2.67	14363.09
FYM II	VAM I	2790.41	41.97	16.30	4899.05	25.20	2.51	7.72	23561.9
	VAM II	5280.86	24.90	23.77	5408.06	24.68	4.48	7.21	42321.27
	VAM III	2162.33	27.43	19.05	4338.36	25.11	9.62	5.38	15547.22
	Control	3496.88	26.65	24.20	5683.38	26.30	7.67	8.49	1649.323
Main effects									
Inoculum		***	***	***	***	***	***	***	***
FYM		***	ns	ns	***	ns	ns	***	ns
Interaction									
FYM X Inoculum		***	***	***	***	***	***	***	***

Table 4.14 Heavy metal profile of rhizospheric ash of *Vetiver zizanoides*

Treatment		*Concentration (mg/kg)*							
		Fe	Pb	Ni	Al	Cr	As	Cd	Si
FYM I	VAMI	19759.82	17.17	24.59	10677.96	43.04	20.22	3.46	138495.40
	VAM II	18302.08	9.279	23.23	6306.36	25.51	20.44	11.72	151023.90
	VAM III	24186.36	11.55	22.89	8303.78	55.64	35.19	11.78	172495.60
	Control	18833.12	11.83	32.17	8186.17	59.45	42.25	5.32	128463.00
FYM II	VAM I	20527.10	11.02	28.84	11168.21	50.64	20.91	4.47	145792.00
	VAM II	15835.88	14.22	31.95	9103.46	36.03	43.76	13.67	145942.10
	VAM III	22973.47	14.58	32.67	14166.96	54.07	34.80	19.84	153490.50
	Control	16356.41	26.97	32.29	10439.71	71.91	18.99	10.29	158978.70
Main effects									
Inoculum		***	***	***	***	***	***	***	***
FYM		ns	***	***	***	***	ns	***	Ns
Interaction									
FYM X Inoculum		***	***	***	***	***	***	***	***

Colonization data

Table 4.5a Assessment of percentage colonization by VA-mycorrhiza on roots of three herbaceous plant species grown on an ash pond, at Super Thermal Power Station, Korba

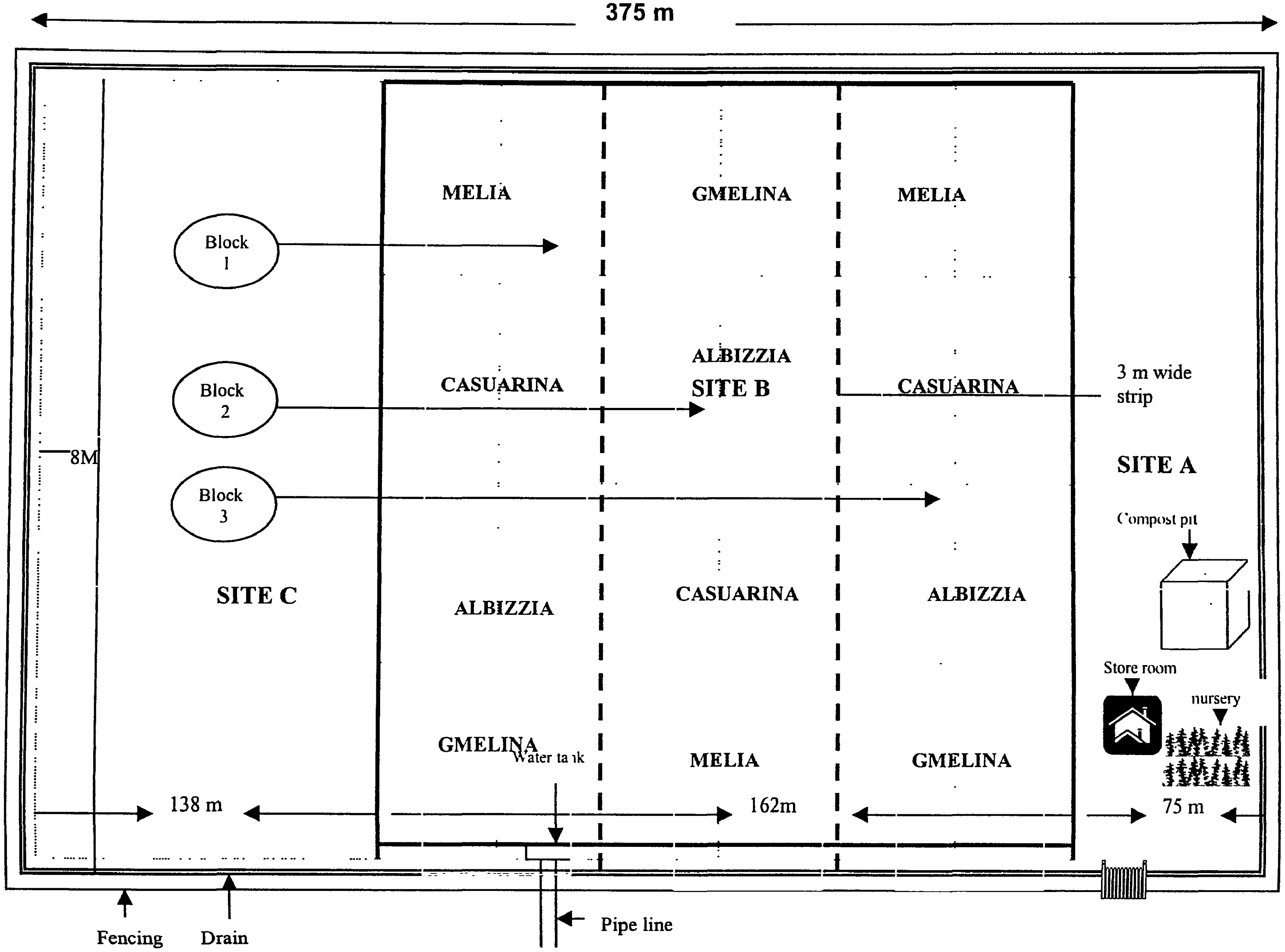
Inoculation Level	Plant species					
	Sesbania sesban		*Mentha arvensis*		*Vetiver zizanoides*	
	Percentage colonization					
	FYM I	FYM II	FYM I	FYM II	FYM I	FYM II
VAM I	58.03 a	57.86 ab	45.8 b	46.86 b	58.26 a	47.46 a
VAM II	52.43 b	55.53 ab	52.93 a	50.43 b	54.53 ab	56.13 a
VAM III	43.43 c	49.83 b	49.30 ab	56.40 a	54.33 ab	50.06 a
LSD (0.05)	3.83	9.16	6.34	5.63	5.69	8.70

Table 4.5b Assessment of percentage colonization by VA-mycorrhiza on roots of three herbaceous plant species grown on an ash pond, at Super Thermal Power Station, Korba

Inoculation Level	*Plant species*		
	Sesbania sesban	*Mentha arvensis*	*Vetiver zizanoides*
	Percentage colonization		
VAM I	57.95 a	46.33 b	52.86 ab
VAM II	53.98 a	51.68 a	55.33 a
VAM III	46.63 b	52.85 a	52.2 ab
Main effects			
Inoculum	***	**	ns
FYM	**	Ns	*
Interaction			
FYM x Inoculum	ns	*	ns

The results were presented at International Mycological Congress (IMC 7) at Oslo, Norway between the period 11 – 17 August 2002. (Annexure 3a)

Figure: 1 Lay Out For STPS, Korba Site



5

Task 4 – Large-scale production of mycorrhizal inoculum for nurseries and mycorrhizal seedlings for plantation

TERI - India

Experiment 1 - Response of AM fungi to different ashes in vitro

Basis of Selection

VA the mycorrhizal isolate, *Glomus intraradices* was selected as test organism on the basis of its survivability, adaptability and higher yield in various plants in the trials conducted at two sites on ash dumps in the past few years.

Materials and methods

Three variants of fly ash dry ash, pond ash, and bottom ash were considered. 1% of these fly ash types were used in the medium.

Host : Carrot
Fungal strain : *Glomus intraradices*
Media : Modified White' s media
Experimental conditions: The system was kept in the dark at 28°C

Parameters analyzed:

Following parameters were analyzed in the experiment:

- Root Length : Roots were characterized into four categories based on their thickness as primary, secondary, tertiary, finer tertiary (tert II), (Figure 5.1)
- Percent root length colonization (Figure 5.2)
- pH at zero and harvest time (Figure 5.4)
- Total spore production (Figure 5.3)
- Mycelial biomass (Figure 5.3)
- Elemental analysis of media for Cu, Mn, Pb, Cr, Cd, Zn, Fe and Ni (Table 5.1 and 5.2)

The results revealed that the presence of dry ash stimulated fungal growth. Similar results were also observed with pond ash, however to a little lesser extent. The presence of bottom ash significantly affected fungal growth as the spore formation, hyphal biomass, and mycorrhizal colonization was reduced to minimum (Figure 5.1- 5.3). The colonization percentage was highest in dry ash followed by pond ash and the control set and was least in the bottom ash indicating the survivability and spread of the fungus in two of the other ash variants.

Under conditions where ash amendments were not given, (control) root biomass was significantly higher showing the normal increasing trend of different root growth stages i.e., primary, secondary, tertiary and finer tertiary (tertiary II). Unlike the fly ash-treated sets showed a drop in the tertiary phase of root growth. However, in bottom ash a decreasing trend was followed soon after an increase in the secondary roots, suggesting that this form of ash had some significant inhibitory effect on the root system as well (Figure 5.1, 5. 2). These observations reveal that bottom ash is highly detrimental for the growth and development of mycorrhiza and the plant system as well.

Figure 5.1 Comparative details of root length profile and colonization % of ash-amended media

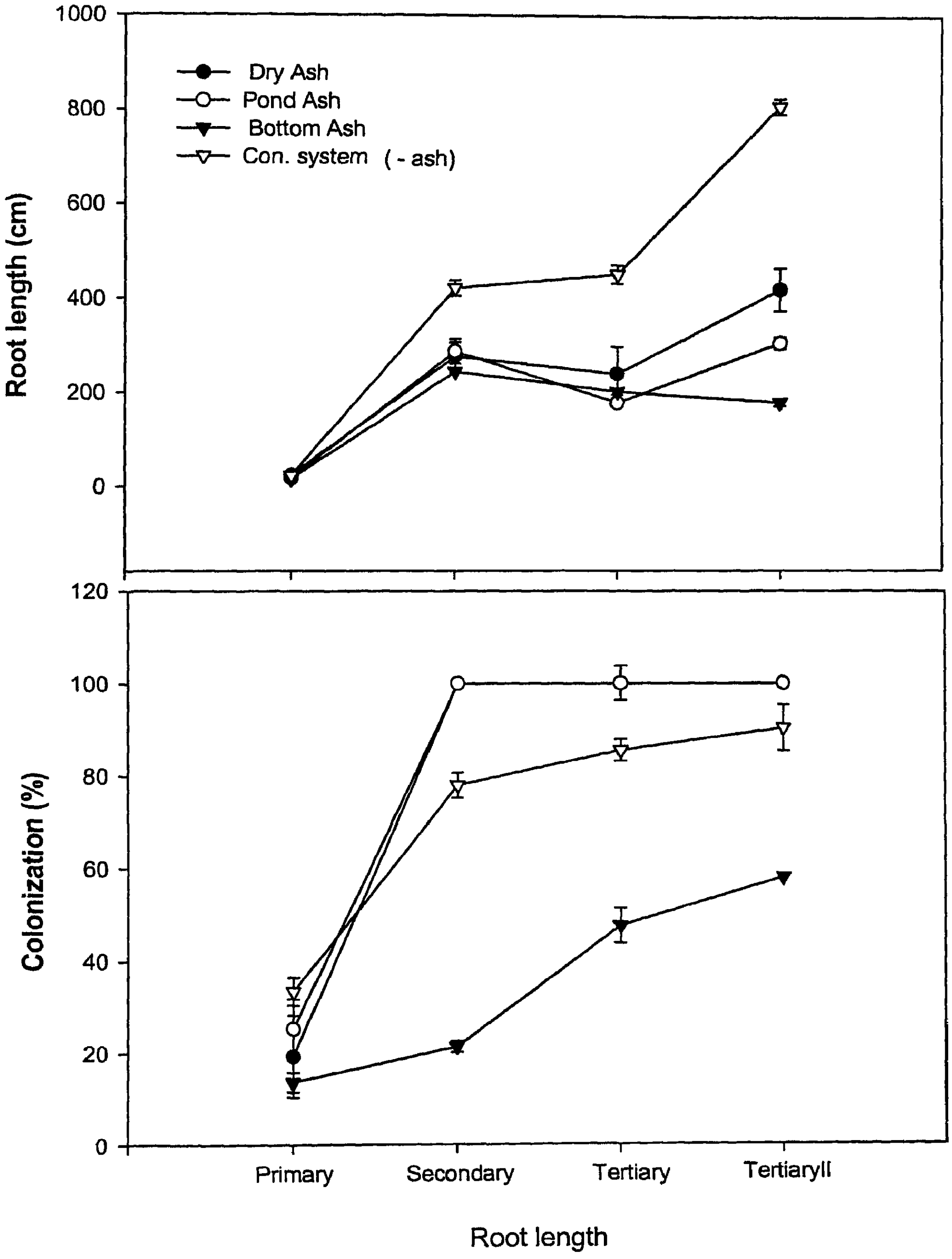


Figure 5.2: Comparative details of total root length and colonization % of ash-amended systems

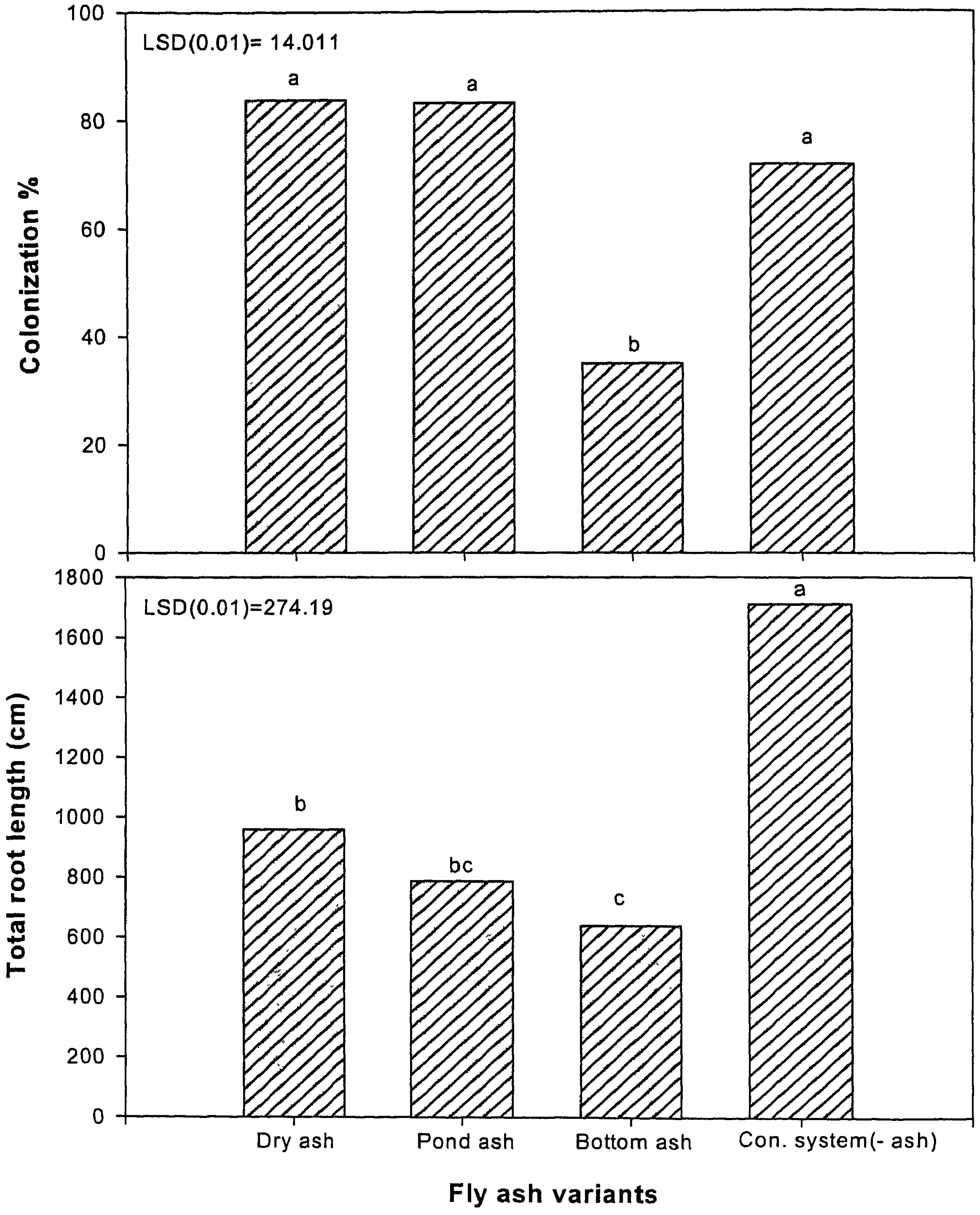


Figure 5.3: Comparative details of total spore count and mycelial biomass in fly ash-amended media

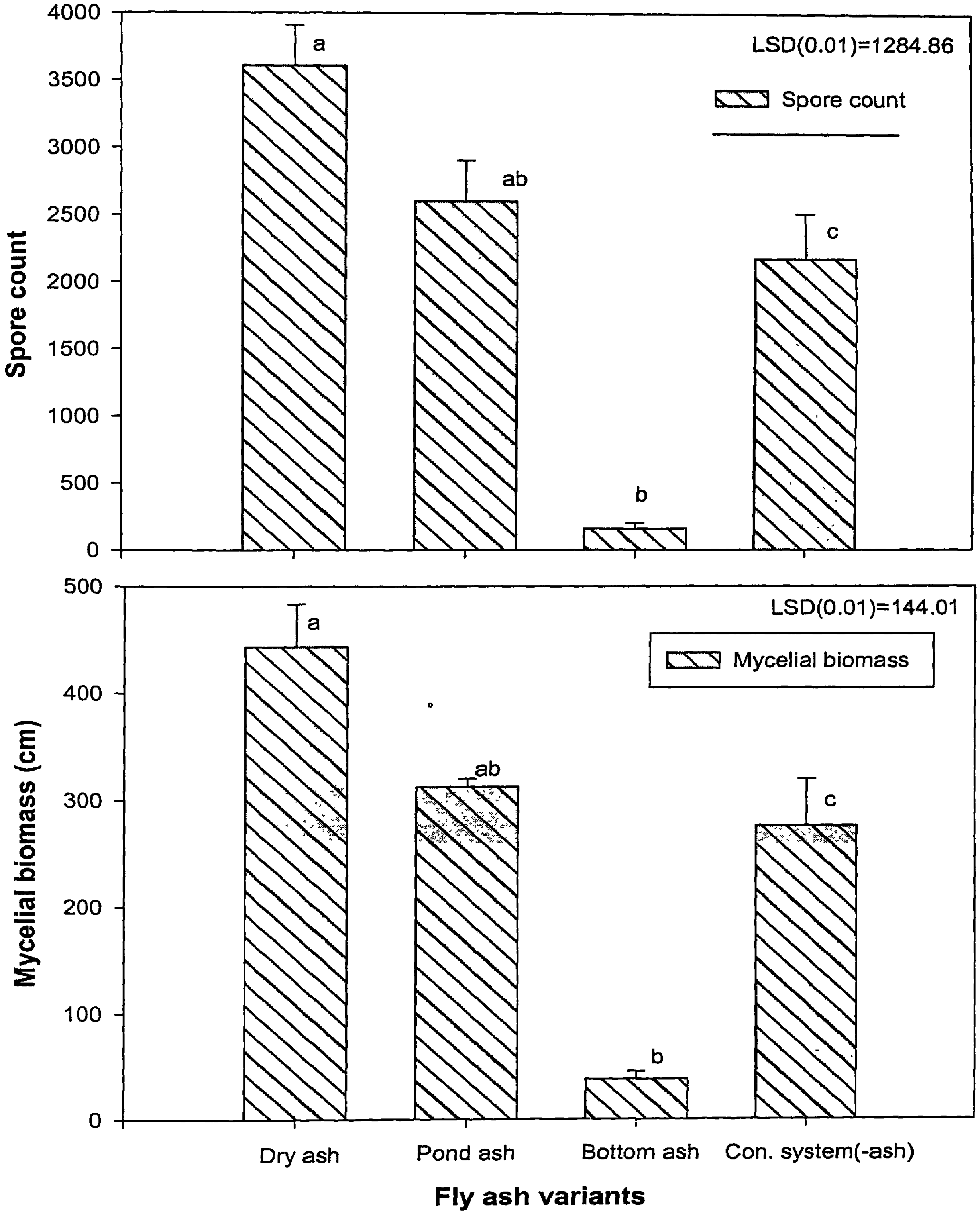


Figure 5.4: pH in different ash-amended media

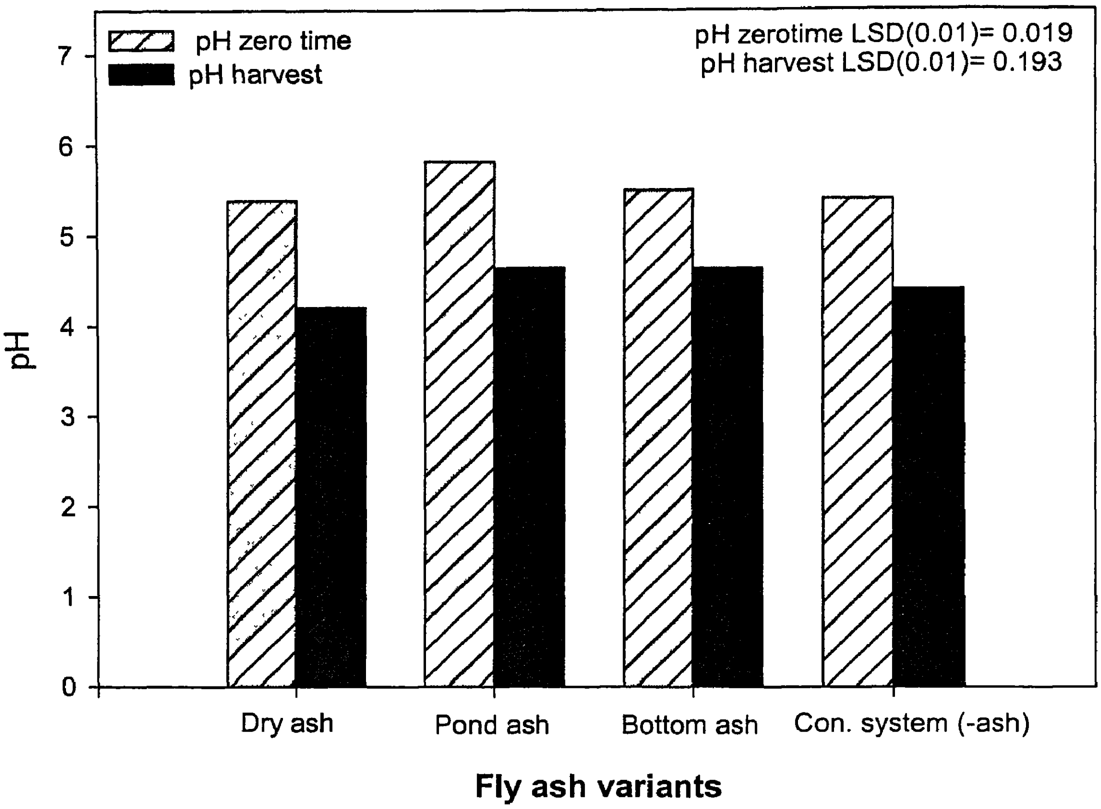


Table 5.1 Trace element analysis of ash-amended media

Elements	Media + Dry ash	Media + Pond ash	Media + Bottom ash	Con. Media (-ash)
Mn (ppm)	1.63	1.84	1.86	BDL
Pb (ppm)	1.42	1.89	1.78	BDL
Cr (ppm)	1.42	1.55	1.25	BDL
Cd (ppm)	0.6	0.62	0.6	BDL
Ni (ppm)	1.35	1.6	1.28	BDL
Fe (ppm)	13.4	21.8	7.1	BDL
Cu (ppm)	4.09	2.12	2.70	0.028
Zn (ppm)	1.88	2.47	1.82	BDL

Table 5.2 Comparison of chemical properties of different ashes

Elements	*Pond ash*	*Bottom ash*	*Dry ash*
PH	7.1	7.48	6.31
EC (ds/m)	2.59	0.2	0.35
Organic carbon %	3.8	1.24	1.83
Available P (ppm)	0.65	0.05	0.31
Nitrogen %	0.357	87.11	0.238
Available K (ppm)	36.33	147.33	43
Total Fe (ppm)	41926	42756.3	27540
Total Zn (ppm)	76.66	105.55	96.66
Total Mn (ppm)	598.11	691.3	69.3
Total Cr* (ppm)	438.8	629.5	598.1
Total Ni (ppm)	1307.3	1462.8	1686.22
Total Pb (ppm)	1133.3	1148.1	1296.6
Total Cd (ppm)	248.1	324.05	300.96
Total Cu (ppm)	-	4.06	31

(2nd year)

Experiment 2 – Behavioural aspects of arbuscular mycorrhizal fungi: Glomus intraradices to different coal ash types in vitro.

Introduction

To study the behaviour and response of arbuscular mycorrhizal fungi *Glomus intraradices* to different ash types at higher concentrations (5 & 50%) under *in vitro* (ROC) system.

Material and methods

AM fungi: *Glomus intraradices*
Transformed root culture: Carrot root (*Daucus carrota*)
Types of ash: ESP ash, bottom ash and pond ash

Experimental conditions

Temperature: 28°C in dark
Duration of incubation: twelve weeks

AM fungi *Glomus intraradices* was grown in M media gelled with 0.3% of phytagel amended with 5% of three types of ashes individually. pH was not adjusted after the amendments and was recorded. Stubs of inoculum containing spores and colonized roots were sub-cultured in ash-amended media. Controls were set with blank roots without AM fungi. Blank media without fungi and spores and media without ashes both blank, with roots and with root and fungi both were also taken as controls. Three replicates were maintained for each of the treatments.

Parameters analyzed

pH at zero time and harvest (Figure 5.9)
Spore count (Figure 5.8)
Mycelial biomass (Figure 5.8)
Mycorrhizal colonization percentage
Elemental absorption in roots (Table 5.3) (Figure 7)
Elemental absorption in hyphae and spores (Figures 5.6, 5.7, Table 5.8)
Elemental status of media (Tables 5.5 and 5.6)
Analysis of ash used in the experiment (Table 5.4)

Results and discussion

The findings reveal that the fungal behaviour varied with different ash treatments. As in the previous experiment, the spore formation and mycelial length was maximum in ESP ash followed by pond ash but least in bottom ash. The same pattern was observed with the root length profile and mycorrhizal colonization percentage. The elemental absorption in roots however did not vary much (Table 5.3, 5.5). However the nutrient uptake profile and elemental absorption by the fungi varied significantly in all the treatments. Maximum absorption of trace and heavy metals in the fungi was observed in the treatment with bottom ash as compared to others. The reason of the poor spread and proliferation of the fungi in bottom ash could be its capacity to absorb more trace and heavy metals. This is interesting as the same fungal isolate behaved differently in its absorption capacity. The possible reason could be the difference in the cation exchange capacity.

Table 5.3 Chemical analysis of trace elements in roots of 5% different ash-amended media

Treatment	*Zn*	*Cu*	*Fe*	*Pb*	*Cd*	*Co*	*Mn*	*Cr*	*Ni*
ESP ash +VAM	4.965	40.62	1.473	39.903	0.352	0.298	8.427	2.327	0.718
ESP ash -VAM	12.63	39.175	1.5115	24.145	0.122	0.221	9.838	1.289	1.250
Pond ash +VAM	13.074	51.03	1.274	73.872	0.153	0.218	9.550	3.015	1.838
Pond ash-VAM	14.725	36.76	0.742	33.54	0.087	0.665	10.06	2.634	1.108
Bottom ash +VAM	5.275	54.501	1.568	23.748	0.169	0.315	10.22	2.928	1.915
Bottom ash -VAM	12.114	50.53	0.433	22.126	0.103	0.154	10.089	1.703	1.176
Con. System +VAM	11.951	33.105	0.047	19.824	0.118	0.056	8.991	0.68	0.7
Con. System -VAM	14.268	118.07	22.790	0.058	0.093	0.031	3.0955	0 408	3.855

Figure 5.5: Elemental analysis of roots grown under different ash-amended media with and without AMF

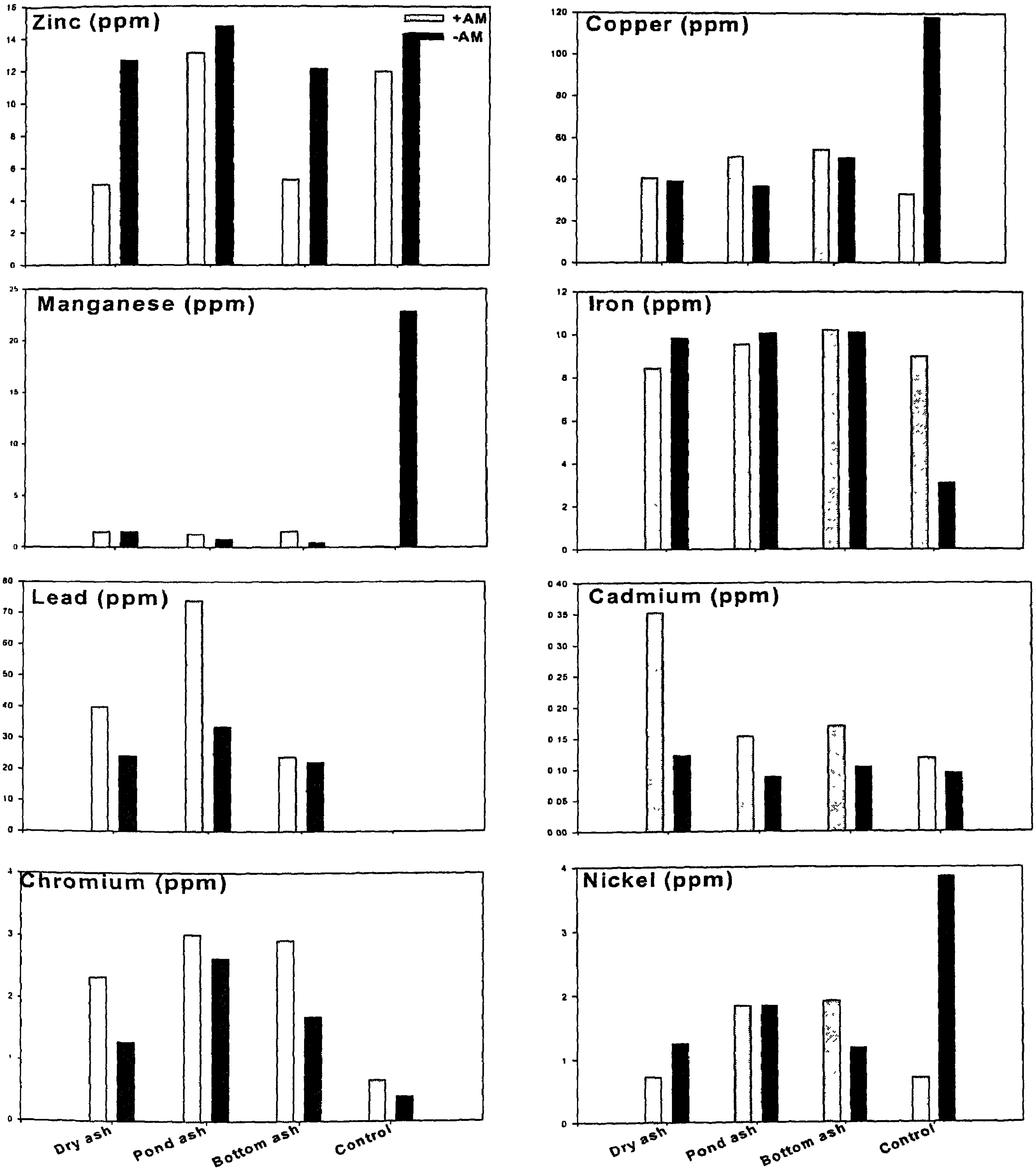


Figure 5.6: Elemental analysis of mycelia and spores of Glomus intraradices exposed to different ash amended media

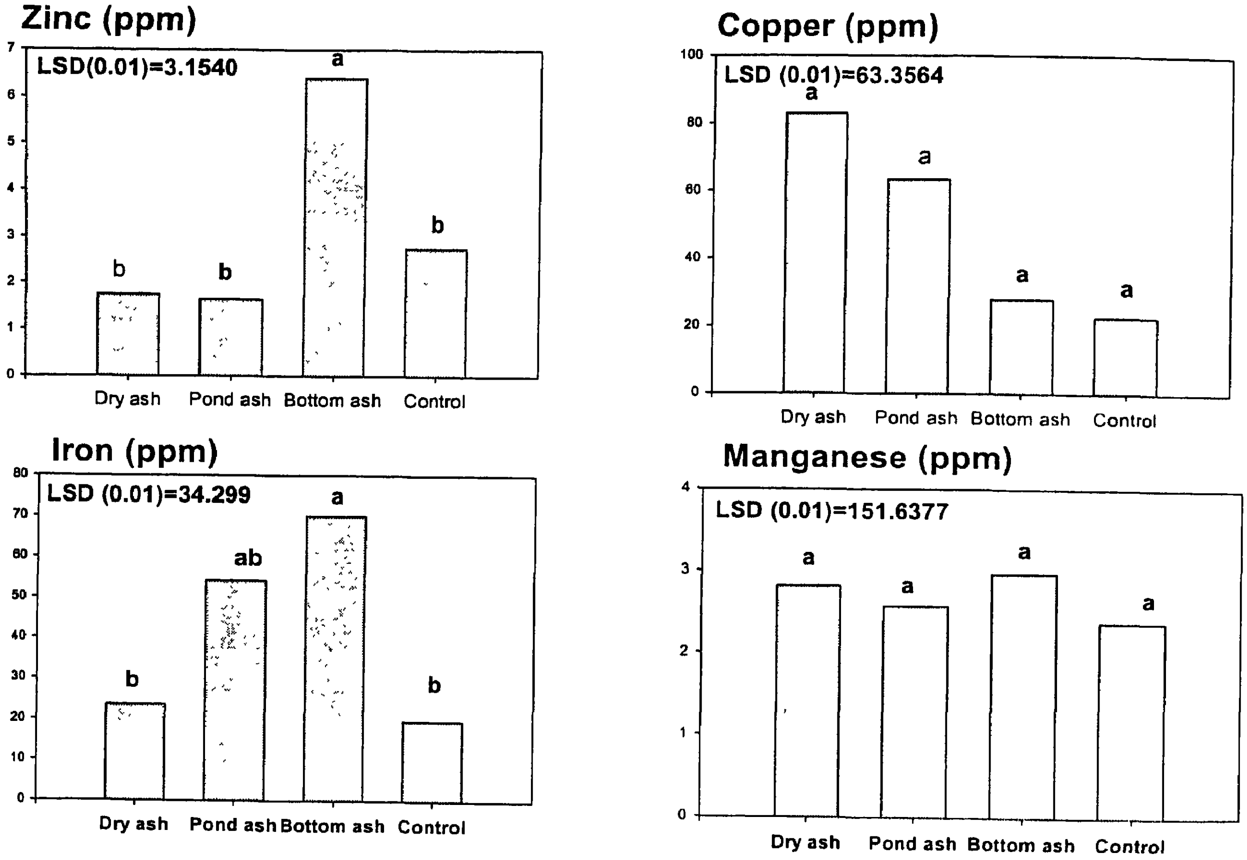


Figure 5.7: Elemental analysis of mycelia and spores of Glomus intraradices exposed to different ash amended media

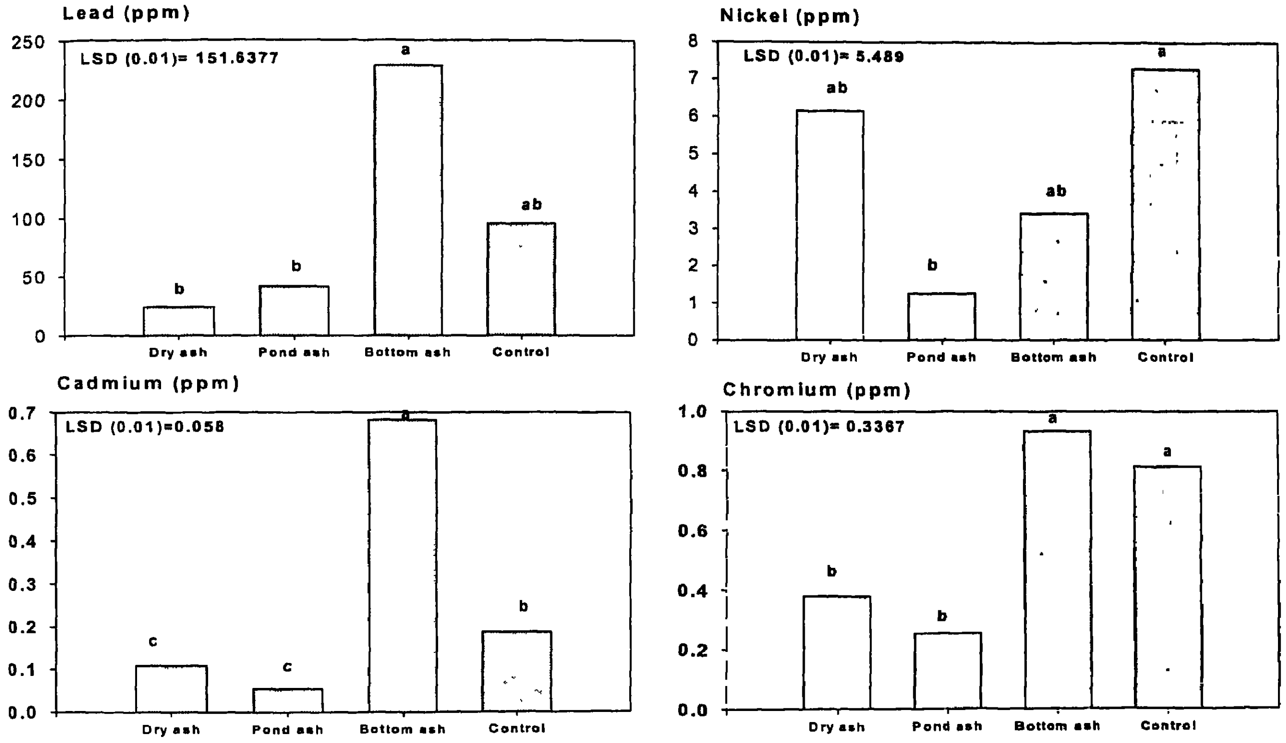


Figure 5.8 Comparison of spore count and mycelial biomass in three different ash amended media

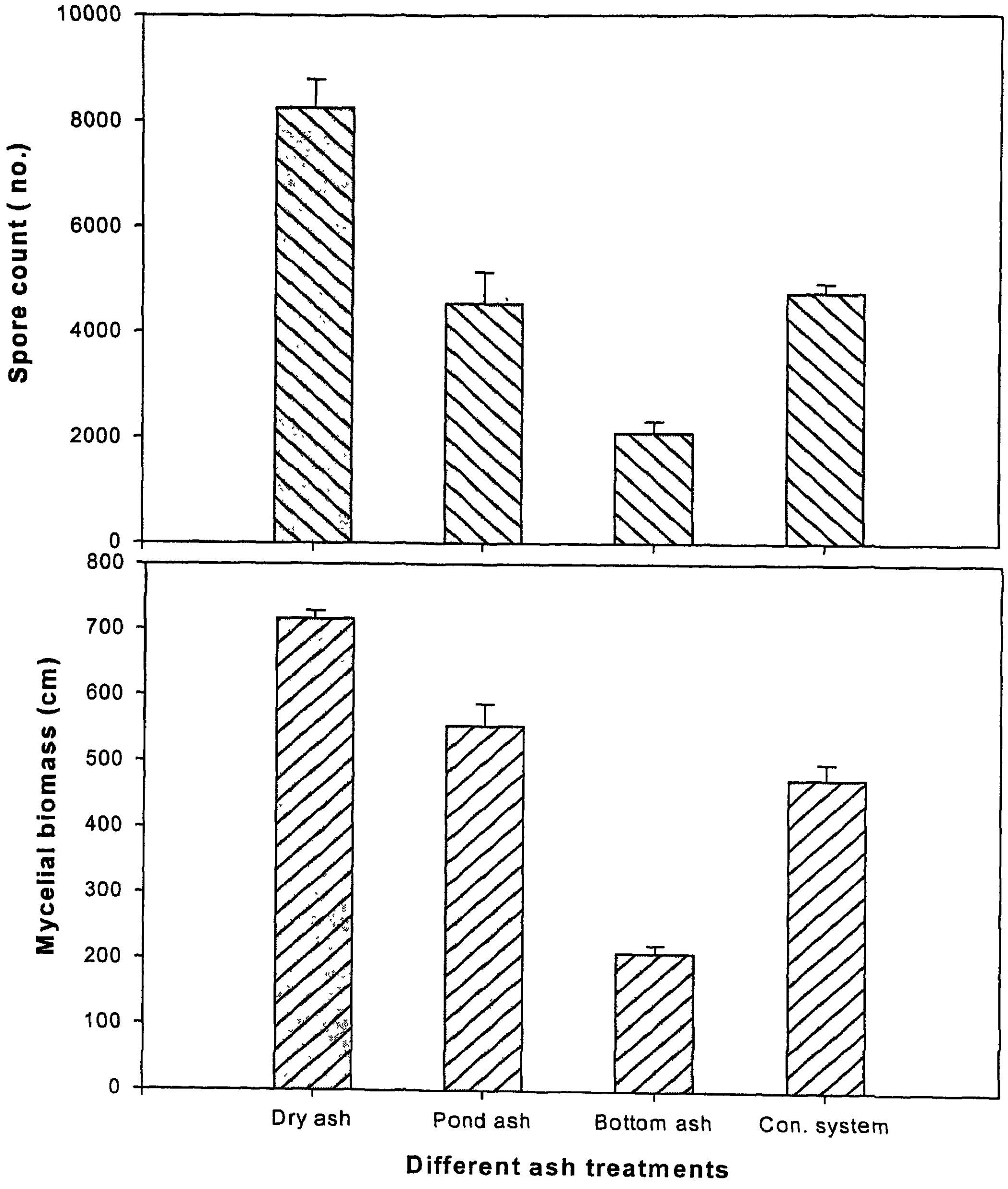


Figure 5.9: pH change in different 5 % ash amended media with and without AMF

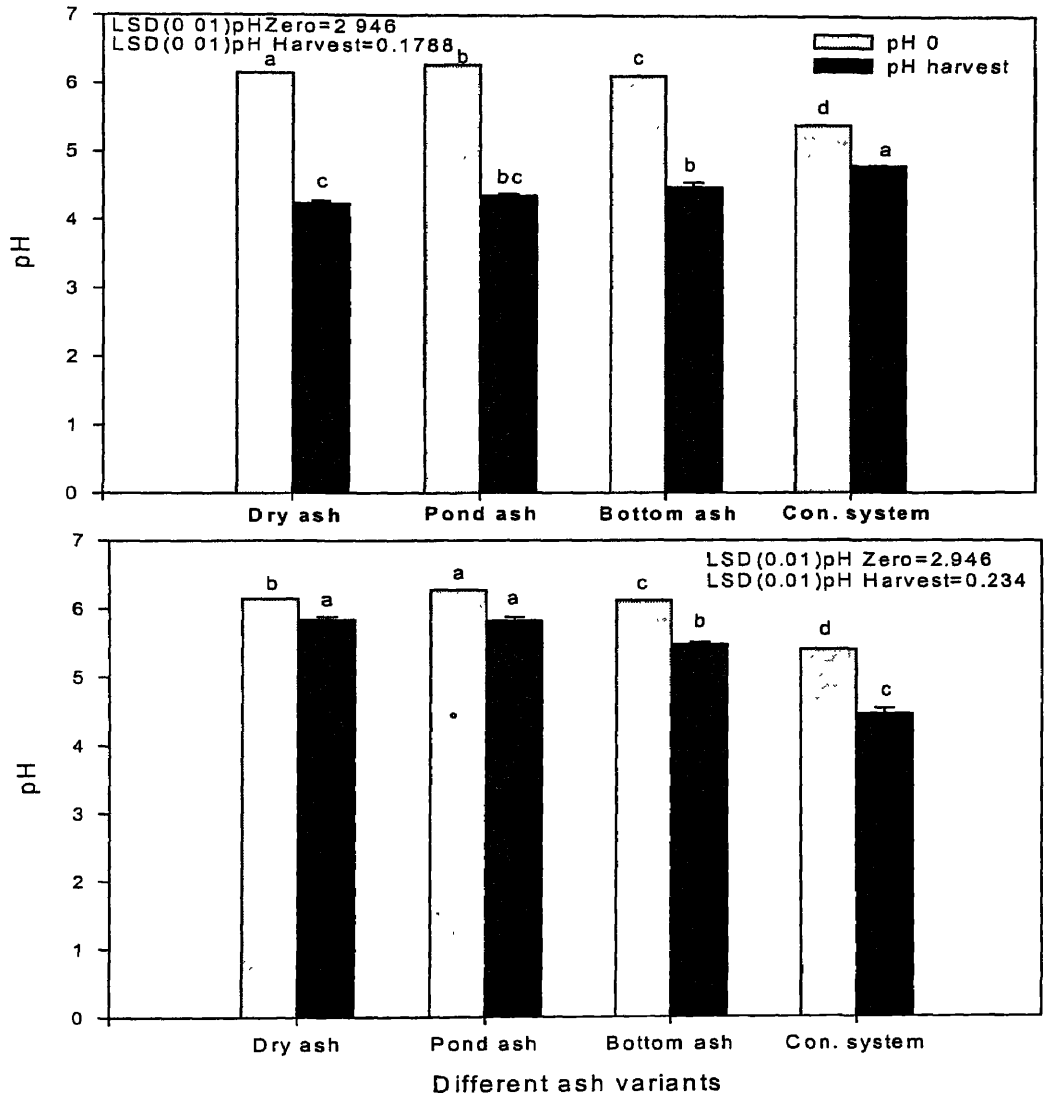


Table 5.4 Comparative details of physico-chemical properties of three ashes used in the ash amendment experiment

Physico- chemical properties		*ESP ash*	*Pond ash*	*Bottom ash*
Particle size distribution g/kg	< 2 microns	47	32	57
	2/20 microns	447	285	317
	20/ 50 microns	301	384	351
	50/ 200 microns	193	282	275
	200/ 2000 microns	12	17	0
PH		5.3	6.9	9.3
Cation exchange capacity (CEC) Cmolc /g of ash		0.4	0.4	1.0
Electrical conductivity (EC)		0.48	0.2	0.28
The values of elements are in ppm				
Zinc (Zn) Total HF		135.0	90.1	50.3
Iron (Fe) Total HF		1850	1810	3830
Manganese (Mn) Total HF		168.3	154.7	398.7
Copper (Cu) Total HF		67.5	57.6	45.3
Lead (Pb) Total HF		61.1	40.3	16.9
Nickel (Ni) Total HF		58.0	50.8	39.5
Cobalt (Co) Total HF		23.3	17.0	85.1
Cadmium (Cd) Total HF		0.48	0.26	0.07
Chromium (Cr) Total HF		144.8	134.6	114.7
Boron (B) Soluble		0.46	0.08	BDL
Sodium (Na) Total HF		30	30	30
Aluminum (Al) Total HF		13240	13300	11830
Magnesium (Mg) Total HF		200	190	220
Calcium (Ca) Total HF		220	210	360
Potassium (K) Total HF		710	680	630
Molybdenum (Mo) Total HF		6.70	1.65	1.50
Arsenic (As) Total HF		5.56	BDL	< 0.50

Table 5.5 Comparative details of media analysis of total elemental status of micro, macro and heavy metals from inoculation with roots and mycorrhiza, only roots and without inoculation in all three ash amendments

Ash type	*Treatment*	*Zn (ppm)*	*Mn (ppm)*	*Fe ((ppm)*	*Cu (ppm)*	*Cd (ppm)*	*Ni (ppm)*	*Cr (ppm)*	*Pb (ppm)*	*Co (ppm)*
ESP ash	Media + ash (A)	16.27	15.91	74.30	38.31	0.399	1.745	2.662	11.581	1.035
	A + roots (B)	7.713	1.31	24.13	15.69	0.017	0.204	0.178	109.25	0.05
	(B) +AMF	2.788	1.30	19.14	14.725	0.05	0.35	0.253	33.95	0.041
	LSD (0.01)	0.338	0.335	6.094	6.796	0.124	0.136	0.349	19.100	0.973
Pond ash	Media + ash (A)	15.61	12.06	78.29	39.23	0.026	3.318	2.212	12.11	0.656
	A + roots (B)	1.879	1.879	18.03	16.29	0.084	0.11	0.154	64.62	0.027
	(B) +AMF	3.296	1.308	11.76	11.76	0.012	0.21	0.377	14.61	0.031
	LSD (0.01)	2.781	0.133	5.129	5.857	0.009	0.508	0.641	9.25	0.118
Bottom ash	Media + ash (A)	15.23	5.59	72.9	37.88	13.36	2.021	3.363	10.99	0.54
	A + roots (B)	3.512	1.313	3.784	14.86	0.0136	0.205	0.105	76.271	0.014
	(B) +AMF	6.433	1.273	3.57	3.577	0.019	0.196	0.830	14.612	0.015
	LSD (0.01)	2.214	0.027	1.268	1.584	0.109	0.03	0.19	6.029	0.036
Media without ash	Media without ash (A)	10.926	2.423	20.65	13.536	0.026	2.016	0.167	2.449	0.123
	A + roots (B)	12.86	2.977	21.71	11.62	0.095	2.683	0.37	41.42	0.012
	(B) + AMF	1.456	0.826	0.711	9.08	0.0161	0.080	0.117	19.813	0.007
	LSD (0.01)	4.903	1.591	15.11	13.45	0.113	1.697	0.246	4.632	0.014

Table 5.6 Comparative details of media analysis of available elemental status of micro, macro and heavy metals from inoculation with roots and mycorrhiza, only roots and without inoculation in all three ash amendments

Ash type	*Treatment*	*Zn (ppm)*	*Mn (ppm)*	*Fe ((ppm)*	*Cu (ppm)*	*Co (ppm)*	*Ni (ppm)*	*Cr (ppm)*	*Pb (ppm)*
ESP ash	Media + ash (A)	0.008	0.184	4.183	0.644	0.003	0.462	2.192	1.348
	A + roots (B)	0.002	0.588	11.9	1.523	0.001	0.033	0.065	4.418
	(B)+AMF	0.008	0.56	11.19	1.018	0.001	0.032	0.067	7.496
	LSD (0.01)	0.001	0.114	3.322	0.192	0.003	0.129	0.123	0.241
Pond ash	Media + ash (A)	0.007	0.363	9.780	1.633	0.003	0.383	1.558	1.091
	A + roots (B)	0.032	0.516	6.963	1.325	0.001	0.386	0.06	3.141
	(B) +AMF	0.008	0.606	6.43	0.448	0.0007	0.040	0.05	4.132
	LSD (0.01)	0.0006	0.626	5.408	0.066	0.001	0.021	0.079	0.759
Bottom ash	Media + ash (A)	0.005	0.191	10.63	0.091	0.0003	0.222	2.191	1.348
	A + roots (B)	0.03	0.66	0.583	1.396	0.001	0.188	0.062	1.763
	(B) +AMF	0.12	1.046	0.573	1.020	0.001	0.057	0.061	2.633
	LSD (0.01)	0.013	0.218	0.075	0.141	0.0096	0.242	0.145	0.288
Media Without ash	Media without ash (A)	0.0061	0.163	0.998	0.271	0.001	0.052	0.005	0.508
	A + roots (B)	0.896	0.446	0.522	1.355	0.001	0.002	0.156	4.756
	(B) + AMF	0.032	0.570	0.518	1.356	0.001	0.036	0.105	5.167
	LSD(0.01)	1.536	0.154	0.115	0.244	0.001	0.016	0.088	0.372

Table 5.7 Comparative details of total elemental status in roots with and without mycorrhiza of micro, macro and heavy metals from inoculation with roots and mycorrhiza, only roots and without inoculation in all three ash amendments

Ash type	*Treatment*	*Molybdenum (ppm)*	*Arsenic (ppm)*	*Boron (ppm)*	*Aluminium (ppm)*	*Magnesium (ppm)*	*Calcium (ppm)*	*Potassium (ppm)*	*Sodium (ppm)*
ESP ash	Un-colonized	0.898	0.079	1169.67	1970.61	714.21	2661.12	368.33	1663.14
	Colonized	0.722	0.15	383.84	2364.59	113.35	862.57	549.79	1818.69
	LSD (0.01)	0.74	0.05	369.93	485.41	65.47	646.68	273.56	548.39
Pond ash	Un-colonized	0.44	0.06	1198.93	2236.9	591.33	1557.74	331.85	1709.26
	Colonized	0.139	0.15	603.66	386.74	757.95	992.36	593.61	1404.98
	LSD (0.01)	0.12	0.04	442.44	769.67	150.42	570.11	30.62	192.69
Bottom ash	Un-colonized	842.94	149.71	1165.11	1179.03	653.69	1743.70	211.84	2198.32
	Colonized	1.592	0.52	600.71	2750.56	115.12	1254.4	408.73	2829.88
	LSD (0.01)	769.87	67.05	289.04	314.01	93.91	1014.8	52.21	481.98
Media without ash	Un-colonized	0.80	0.067	838.48	378.31	361.94	967.93	1656.41	893.20
	Colonized	0.56	0.10	700.52	348.15	456.17	860.22	2451.51	759.85
	LSD(0.01)	0.15	0.06	55.19	119.84	278.19	132.93	1387.36	340.45

Table 5.8 Comparative details of total elemental status in extramatrical AM fungal biomass of micro, macro and heavy metals from inoculation with roots and mycorrhiza, only roots and without inoculation in all three ash amendments

Trace elements & heavy metals (ppm levels)	*Extramatrical mycorrhizal biomass from ESP ash media*	*Extramatrical mycorrhizal biomass from pond ash media*	*Extramatrical mycorrhizal biomass from bottom ash media*	*Extramatrical mycorrhizal biomass from media without ash*
Zinc (Zn)	2.10 ± 0.15	1.644 ± 0.26	6.38 ± 0.962	2.74 ± 0.33
Copper (Cu)	114.64 ± 11.91	63.59 ± 13.91	28.03 ± 0.68	25.80 ± 5.14
Iron (Fe)	23.9 ± 1.55	54.12 ± 12.15	70.10 ± 1.02	26.59 ± 3.34
Manganese (Mn)	2.82 ± 0.09	2.57 ± 0.002	2.97 ± 0.23	2.37 ± 0.31
Lead (Pb)	24.45 ± 2.42	42.15 ± 8.78	228.50 ± 50.75	95.57 ± 3.72
Cadmium (Cd)	0.139 ± 0.01	0.055 ± 3.17	0.68 ± 0.01	0.186 ± 0.02
Cobalt (Co)	0.05 ± 0.01	0.03 ± 0.001	0.05 ± 0.01	0.12 ± 0.04
Chromium (Cr)	0.37 ± 0.008	0.27 ± 0.001	0.93 ± 0.08	0.81 ± 0.06
Nickel (Ni)	5.82 ± 1.39	1.24 ± 0.27	3.43 ± 0.31	7.28 ± 1.31
Sodium (Na)	2170.72 ± 342.8	130.44 ± 8.27	152.75 ± 25.42	320.64 ± 71.55
Aluminium (Al)	187.09 ± 28.66	76.07 ± 12.71	300.97 ± 11.80	156.98 ± 36.54
Magnesium (Mg)	486.70 ± 96.95	75.00 ± 5.62	162.12 ± 23.74	59.06 ± 7.64
Calcium (Ca)	1484.69 ± 210.76	635.42 ± 283.15	1905.00 ± 181.98	400.72 ± 72.4
Potassium (K)	99.23 ± 10.96	131.41 ± 23.83	257.54 ± 54.21	121.04 ± 20.44
Arsenic (As)	0.093 ± 0.03	0.087 ± 0.001	0.469 ± 0.028	0.073 ± 0.016
Boron (B)	127.20 ± 3.48	376.56 ± 67.07	249.89 ± 42.85	123.62 ± 1.22
Molybdenum (Mo)	0.26 ± 0.03	0.06 ± 0.02	0.263 ± 0.06	0.327 ± 0.005

The results were presented at International Mycological Congress (IMC 7) at Oslo, Norway between the period 11 – 17 August 2002. (Annexure 4f)

Experiment- 3

(3rd year)

The experiment was designed in continuation with the one in the second annual report to study the response in various ash amendments in media with respect to the excretion of different organic acids in media and total carbohydrate secretion in the media inoculated with *Glomus intraradices*.

Experimental design

Media with 5 % ash amendment (using three types of fly ash – ESP ash, pond ash, bottom ash) and media without ash.

Arbuscular mycorrhizal fungi – *Glomus intraradices*

Duration of incubation– three months

Root cultures – carrot transformed roots

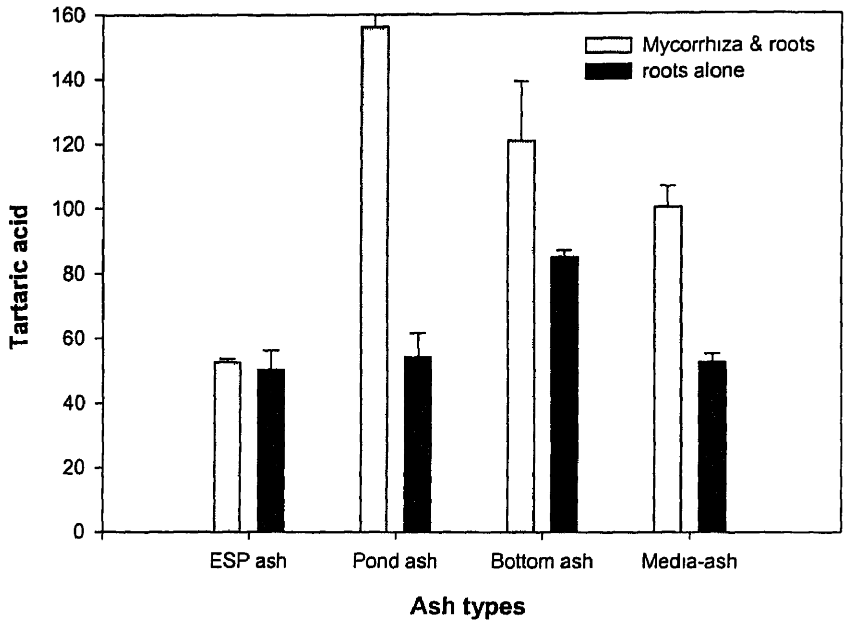
The media after the incubation was harvested by a de-ionization buffer. The ratio of media and buffer was maintained constant at 1:0.5. The samples were then analyzed using the Anthrone method for total carbohydrates using HPLC.

For the organic acid secretion standards of organic acids such as oxalic acid, citric acid, malic acid, succinic acid, tartaric acid, and acetic acid were taken. The samples were then analyzed using Organic acid column (Bio-rad), Aminex ^{R}, HPX-87H, Ion Exclusion column, 300 mm x 7.8 mm with HPLC instrument for the respective secretions in the media amended with different ashes collected from the Korba fly ash. The figures showing the results are documented and enclosed as annexures.

Results and discussion

The results indicate that the production of these organic acids is influenced by the ash amendments. Malic acid appears to be less produced in stress conditions where it is expressed only in the treatment with media without ash. Mycorrhizal roots produced more oxalic acid as compared with non-mycorrhizal roots. Bottom ash treatment produced least oxalic acid. Succinic acid was produced only in non-mycorrhizal roots of media without ash treatment. This indicates that the production of these acids is influenced by the presence of these ashes in media (Figure5.10-5.13). However further investigations are needed to find cause of variation in different treatments.

Figure 5.10 Tartaric acid production in media with different 5 % ash amendments



Fiaure 5.11 Malic acid oroduction in media with amendment

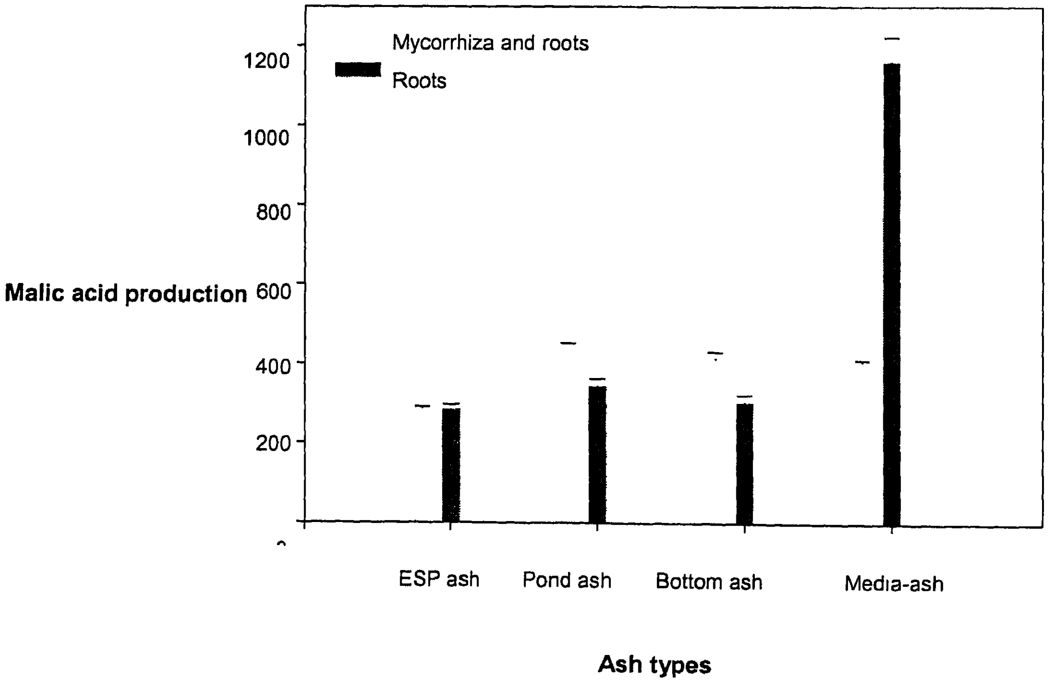


Figure 5.12 Oxalic acid production in media with different 5% ash amendments

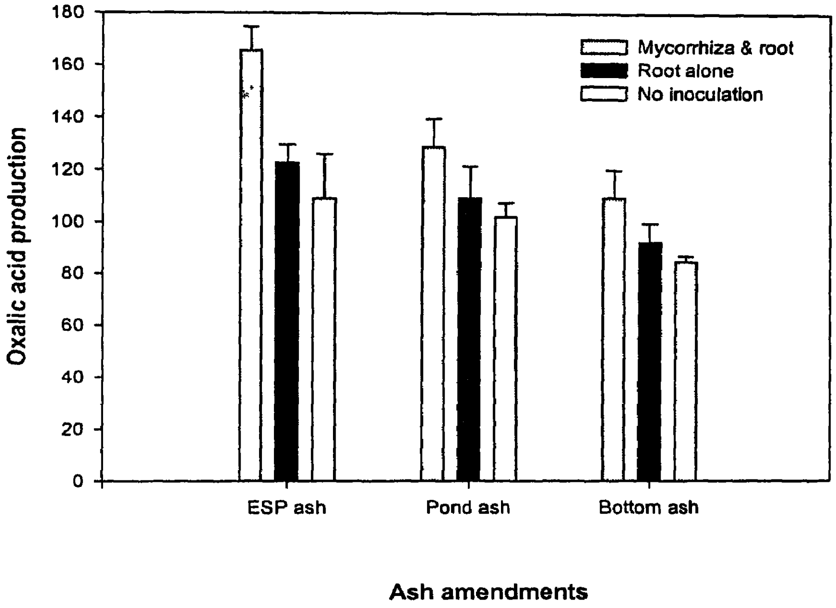
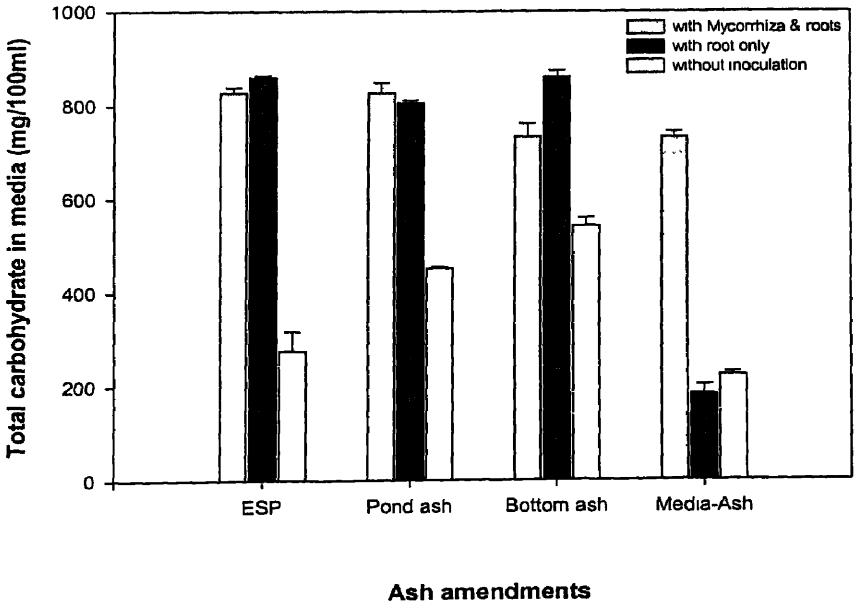


Figure 5.13: Comparative account of total carbohydrate present in media with different 5% ash amendments



Experiment- 4

The experiment was designed to study the variation of host towards response to various ash amendments at higher dosage in media inoculated with *Glomus intraradices.*

Experimental design

Media with 50 % ash amendment (using three types of fly ash- ESP ash, pond ash, bottom ash) and media without ash.

Arbuscular mycorrhizal fungi- *Glomus intraradices.*

Root clones- Five different roots clones; SWA, SWB, SWC, GP (these four of carrot) and clover.

Duration of incubation- Three months.

Parameters analysed-

a. Root biomass estimation using "Tenants formula" (figure 5.15 - 5.17).

b. Mycorrhizal colonisation percentage (Biermann and Linderman, 1981), (figure 5.18).

Results and discussion

The results of the experiment suggests that the mycorrhizal responsiveness and expression in varied ash amended environment *in vitro* was found different in different host-fungal combinations. These hosts belonging to same genus (SWA, B, C, and GP) carrot behave differently in different ash amendments. Unlike previous observations of higher mycorrhizal responsiveness in ESP ash, bottom ash treatment showed better expression of mycorrhiza. The second genus clover also showed difference in expression from the other root clones. Least mycorrhizal spores were observed in bottom ash treatment, a finding similar to lower doses amendments. Thereby indicating that bottom ash environment is stressful to mycorrhiza. Highest expression was observed in treatment where no ash was amended. These observations indicate that mycorrhizal tolerance and expression is host mediated and their adaptability in stressed environment also differs on the basis of the plant system they colonize.

Figure 5.14 Root biomass (cm) of different clonal cultures produced in 50% ESP ash amendment

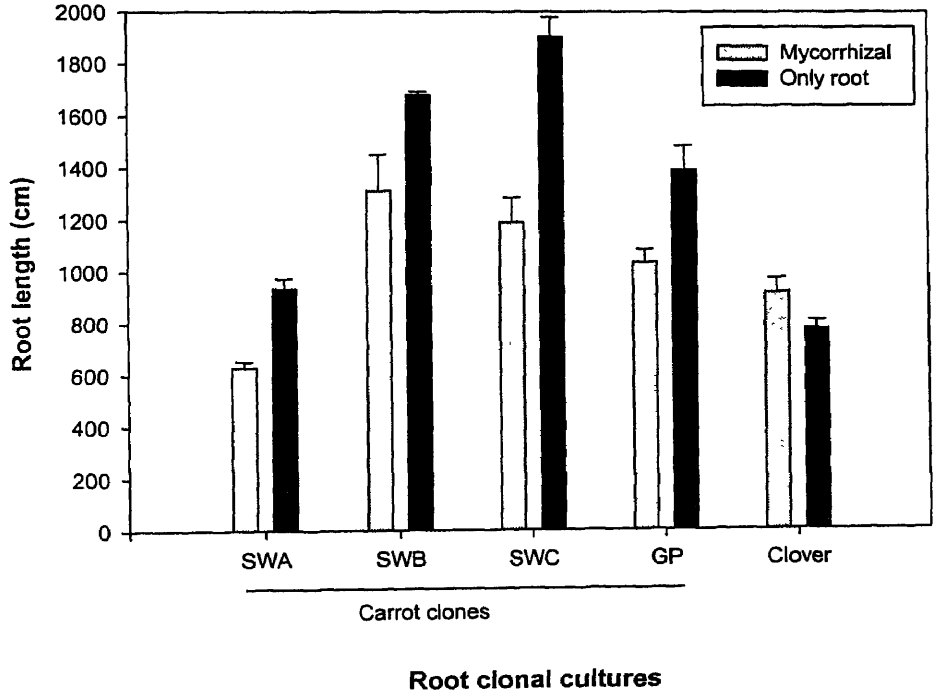


Figure 5.15 Root biomass(cm) produced by different clonal cultures in 50 %pond ash amendment

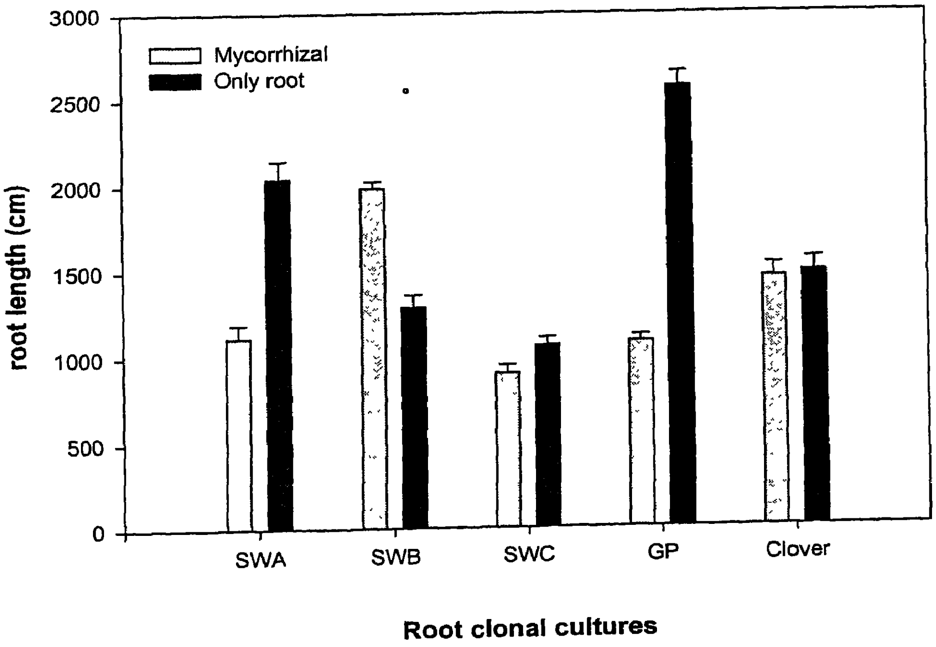


Figure 5.16 Root biomass (cm) prodcued by different clonal cultures in 50% bottom ash amendment

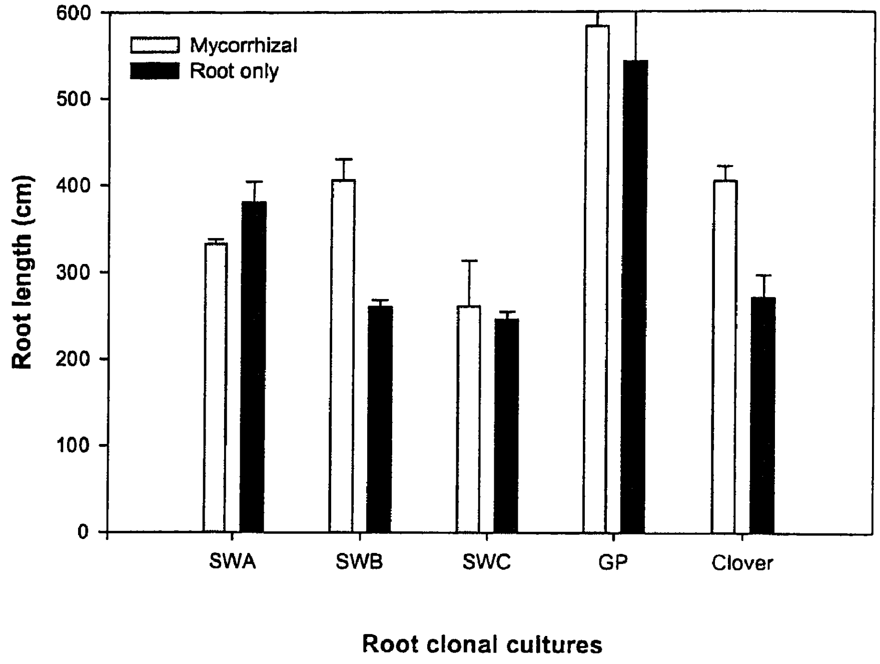


Figure 5.17 Root biomass (cm) produced by different cultures grown in media without ash amendment

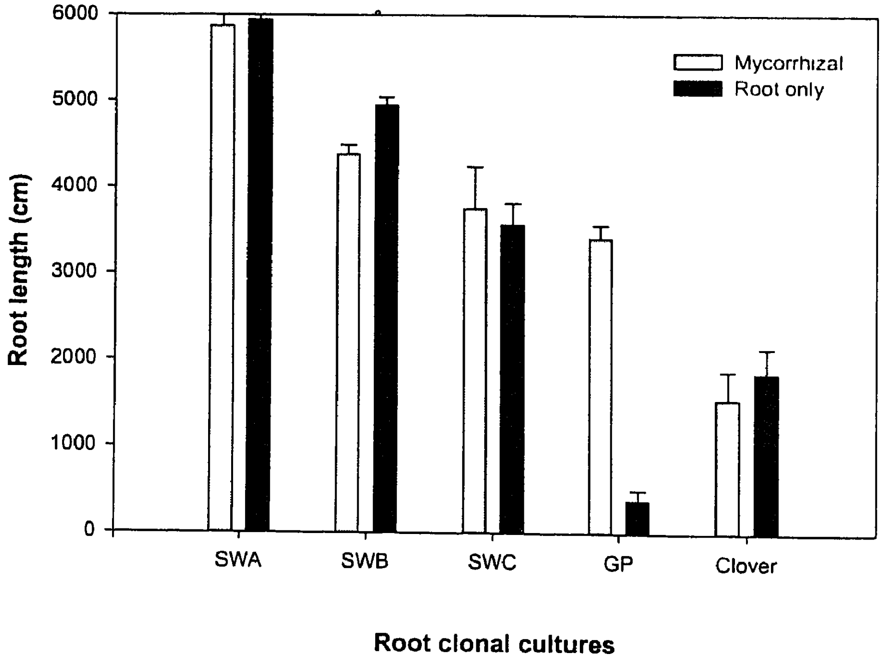
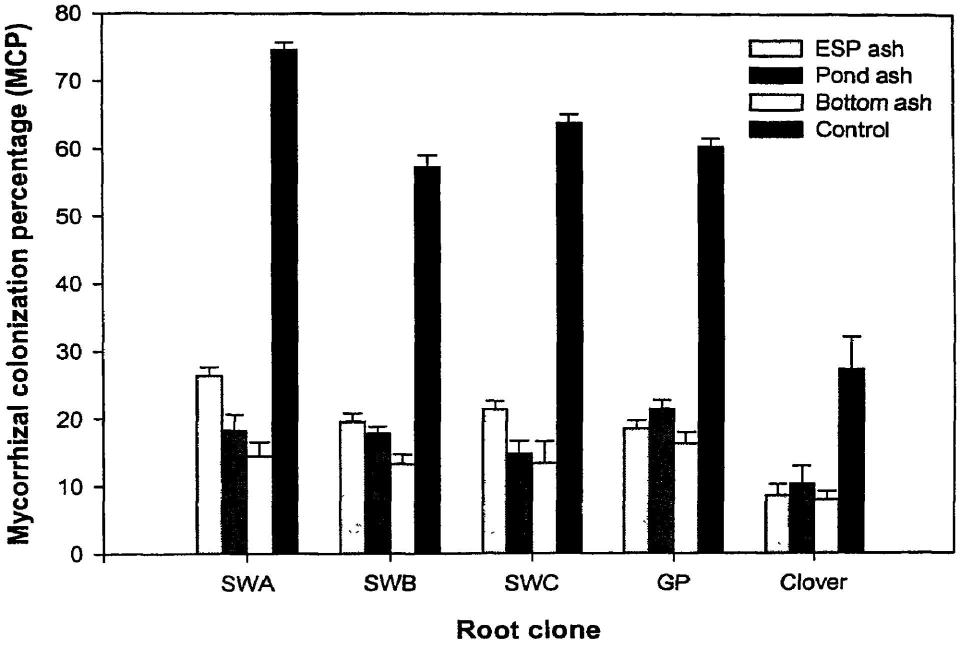


Figure 5.18- Comparative details of mycorrhizal colonisation percentage (MCP) of various host root clones grown in different ash-amended media



Large-scale production

Trees with ectomycorrhizal associations are reported to be benefited by enhanced ion uptake, water uptake, tolerance towards root pathogens and soil toxins. Besides, dramatic improvement in the seedling performance has been observed following introduction of suitable ectomycorrhizal fungi. Thus competent tree seedlings inoculated with compatible fungal partner could act as essential tools for land reclamation disturbed by coal ash discharged from the thermal power stations. Therefore an effective career for mycorrhizal inoculum is necessary for the purpose of mass production in which viability of culture can be effectively maintained and eventually can be introduced in the fields as well.

Materials & Methods

Selection of the isolate

Fungal isolates selected for the experiment are listed below:

Bank Code	Culture Name	Host
EM-1191	*Laccaria laccata*	*Eucalyptus sp.*
EM-1267	*Paxilus involutus*	*Eucalyptus darlympleana*
EM-1212	*Paxilus involutus*	*Populus euramericana*
EM-1235	*Scleroderma flavidum*	*Eucalyptus camaldulensis*
EM-1233	*Scleroderma cepa*	*Eucalyptus sp*
EM-1291	*Pisolithus tinctorious*	*Eucalyptus tereticornis*
EM-1283	*Scleroderma verucosum*	*Shorea roubasta*
EM-1293	*Pisolithus tinctorious*	*Eucalyptus tereticornis*

Preparation of media

Vermiculite and peat were thoroughly mixed in a 2: 1 ratio. The mixture was taken in a glass jar and autoclaved twice for a period of 30 minutes. The substrate was wetted with MMN broth @ half the volume of the mixture. The wetted substrate was again autoclaved for 30 minutes.

Inoculation

The jars after cooling were inoculated with fungal discs cut from the edge of the actively growing fungal colony. The jars were incubated at 24 ^{0}C in a BOD incubator until the mycelium had covered the entire substrate (4 to 6 weeks). Vermiculite-peat based inoculum, when taken directly from the container and applied into the field became rapidly colonized by saprophytic microorganisms. Heavy colonization reduces the effectiveness of the inoculum. Because of this the inoculum was leached with water to remove the non-assimilated nutrients. This eventually increased the inoculum' s effectiveness (Annexure 4 a – c). Synthesis of *Scleroderma flavidum, Laccaria laccata* were also made using different host (Annexure 4d, 4e).

Mass production of arbuscular mycorrhizal fungi in vitro

Large-scale production for subsequent field applications of AM fungi, *Glomus intraradices* under *in vitro* conditions was carried out for bulk production of viable and pure inoculum. This was propagated using *Agrobacterium rhizogenes-* mediated transformed roots of carrot. These roots have a tendency to extensively proliferate which assists mycorrhiza to grow and spread in the medium. Large numbers of propagules were obtained after an incubation of three months. These were used for field applications.

6

Task 5 – Mechanism of adaptation to ash pond environment

TERI – India

In vitro experiments with 5% ash amendment using different types of ashes from the Korba Super Thermal Power Station, on media and subsequent inoculation with mycorrhizal fungi *Glomus intraradices* and transformed roots of carrot was repeated to study their adsorption in the roots and mycelia of *Glomus intraradices*. These samples were harvested after two months of incubation. The samples were analyzed live without disturbing the system using the Scanning Electron Microscopy with EDX attachment.

The results of the SEM, EDX analysis of elemental adsorption on the surface of roots and mycelia are documented in Table 6.1 and 6.2. Pictures of SEM - EDX analysis and the instrument facility are given in Annexure 5a and 5b.

Table 6.1 Levels of various elements adsorbed in the arbuscular mycorrhizal mycelia grown in different ash treatments

Micro, macro and heavy metals (in ppm)	*ESP ash*	*Pond ash*	*Bottom ash*	*Media without ash*
Na	0.164±0.02	0.132±0.01	0.092±0.01	0.303±0.04
Al	0.092±0.01	0.058±0.01	0.065±0.01	0.045±0.004
Si	0.10±0.01	1.16±0.02	0.152±0.07	0.06±0.004
S	0.84±0.05	1.29±0.04	0.84±0.12	0.73±0.04
K	1.3±0.16	2.001±0.19	1.60±2.60	2.60±0.28
Ti	0.03±0.00	0.05±0.01	0.06±0.01	0.04±0.00
Ca	0.98±0.21	1.30±0.14	0.98±0.07	0.87±0.06
Mo	0.07±0.01	0.15±0.01	0.12±0.00	0.34±0.03
Cu	0.13±0.01	0.385±0.18	0.144±0.01	0.11±0.01
Zn	0.08±0.01	0.09±0.01	0.14±0.01	0.12±0.02
Pb	0.15±0.01	0.16±0.02	0.20±0.03	0.23±0.04

The values are the mean of replicates with their standard error of mean. They are in ppm

Table 6.2 SEM Root analysis showing elemental adsorption

Treatments		*Na*	*Al*	*Si*	*S*	*K*	*Ca*	*Ti*	*Cu*	*Zn*	*Mo*	*Pb*
Pond ash Media	Un-colonized	0.17 ±0.01	0.42 ±0.03	0.07 ±0.01	0.49 ±0.15	3.81 ±0.30	0.4 ±0.02	0.06 ±0.008	0.14 ±0.01	0.15 ±0.02	0.30 ±0.04	0.34 ±0.001
No ash media	Un-colonized	1.41 ±0.16	0.11 ±0.01	0.04 ±0.005	2.11 ±0.13	0.66 ±0.03	1.17 ±0.08	0.04 ±0.004	0.13 ±0.02	0.09 ±0.02	0.05 ±0.01	0.27 ±0.01

The values are the mean of replicates with their standard error of mean. They are in ppm

A comparison between the elements in roots and mycelia indicate that certain elements appear more selective to roots (Al and K), while elements like Ca, Cu, Zn appear more selective to fungi. Elements such as S and Si do not seem specific to either. If a comparison is made between un-colonized roots from pond ash and media without ash, they appear similar with respect to selectivity. The elemental adsorption in fungi from all three ashes and from media without ash treatment (**Table 6.1**), suggest that Ca, Mo, Cu and Zn seem to have a greater affinity for fungi.

These observations were compared with data obtained by atomic absorption spectroscopy which supported them.

The above analysis was carried out at INRA, Nancy, France.

Nancy - France

Alteration and immobilization by ectomycorrhizal fungi

Considering the preliminary conclusion from task 2 (absence of heavy metal toxicity) which needs to be confirmed in the field, the emphasis will be on alteration of ashes by ectomycorrhizal fungi and mineral neoformation.

To follow the weathering of ashes in the presence of mycorrhizal fungi and identify the related mechanisms, an *in vitro* experiment is being carried out (running into its third month). Techniques such as X-ray diffraction (Fig. 2), organic acid analysis, electron microscopy, ICP, will be used to analyse the ashes at the termination of the experiment (3 months).

Another experiment is underway in a glasshouse to compare the weathering of ashes by roots of trees (*poplar* and *douglas* fir) associated or not with the mycorrhizal fungus *Laccaria laccata*. The ashes from this experiment will be analyzed as above at the termination of the experiment (6 months).

Finally, root samples have been collected from the Pont à Mousson ash dyke, rhizospheric and mycorhizospheric ashes have been isolated to investigate mineral weathering. The activity of various trees will be compared.

(2nd year)

Some experiments were established to investigate the alteration of ashes by ectomycorrhizal fungi and mineral neoformation. Ectomycorrhizal strains (*Paxillus involutus*, 4 strains; *Pisolithus tinctorius*, 2 strains; *Laccaria laccata*, 1 strain) were grown on agar medium, half of the mycelial colony colonizing ashes according to the protocol described by Paris et al. (Plant and Soil, 1995, 177, 191-201). Nitrogen was supplied as nitrate or ammonium. For technical reasons it was not possible to record as many data as expected from this experiment.

After 3 months growth, the major finding was the presence of calcium oxalate crystals among ashes particles colonized by hyphae (Fig 2a). Calcium oxalate deposition could also be found on the hyphal wall (Fig 2b). These crystals accumulated Ca (microprobe under electron microscope) confirming their identity. The presence of calcium oxalate crystals is the first proof of oxalate excretion by ectomycorrhizal fungi growing on ashes. Since oxalate binds strongly with Ca and Al, it is a very powerful weathering agent. Here the neoformation of Ca-oxalate crystals has unveiled the fungal activity on ashes.

Cation toxicity in plants

Since ash tolerance by ectomycorrhizal fungi does not seems to be a major problem (cf. Task 2) we initiated a programme aimed at understanding the mechanism of cation toxicity in plants, anticipating that it would contribute to the identification of the mechanisms regulating ectomycorrhizal fungi-plant interaction in toxic environments.

There have been many reports suggesting the involvement of reactive oxygen species (ROS) including the superoxide anion ($O_2^-\bullet$) in salt stress. Here we investigated the response of a cell suspension culture of tobacco (*Nicotiana tobaccum* L.; cell line, BY-2) to treatments with various salts of trivalent (including Al), divalent and monovalent metals. The first results indicated a strong relationship between the valence of metals and the level of $O_2^-\bullet$ production. The regulation of $O_2^-\bullet$ production, including by fungi was proposed to be investigated.

(3rd year)
We could not establish a major problem related to heavy metal toxicity in the Korba ashes. The conclusions from Task 2 at the end of 2nd year were as follows:

- The presence of ashes was not hindering the growth of fungal strains tested including isolates characterized as 'heavy metals sensitive'.
- In the ashes the concentrations of heavy metal ions, though high, did not exceed the limits of toxicity set for soils.
- The cations are evenly distributed throughout the ashes. The ash composition seems to be homogeneous.
- All parameters recorded suggest that Ni and Cd bioavailability is low or very low.

However we pointed out that these preliminary conclusions needed to be confirmed by short term and long term field experiments (i.e. aluminium toxicity is highly dependent on pH and could be difficult to predict on a long term, following soil acidification). We therefore initiated a programme aimed at understanding the mechanism of cation toxicity in plants, anticipating that it would contribute to the identification of some mechanisms regulating ectomycorrhizal fungi-plant interaction in toxic environments. This work was extended during the 3rd year.

Mechanism of cation toxicity in plants

Two papers, with the following abstracts, have been published on this topic (the reprints are enclosed in this report – Annexure 5c and 5d).

Ectomycorrhizal fungi could regulate plant response to toxic aluminium concentrations

Having investigated the interactions between cations and plants, including the ways some cations might be regulating the activity of others, we considered ectomycorrhizal fungi, focussing on the ways fungi could interfere with the mechanisms evidenced above. A paper is being prepared regarding aluminium toxicity, the key points are presented hereunder.

Induction of $O_2^- \bullet$ generation in tobacco cell suspension culture by $AlCl_3$ treatment

Addition of $AlCl_3$ to a tobacco cell suspension culture resulted in an immediate burst of $O_2^- \bullet$ production reaching the maximal level within 0.5 sec and lasting for about 15-20 seconds after $AlCl_3$ addition to the cells. Addition of $AlCl_3$ induced the generation of $O_2^- \bullet$ in a dose-dependent manner, the $AlCl_3$-induced production of $O_2^- \bullet$ was optimal at 6.25 µM and $AlCl_3$ concentrations higher than at 6.25 µM were inhibitory (Fig. 1a). Effect of $LaCl_3$ on generation of $O_2^- \bullet$ was also examined for comparison, although $LaCl_3$ induced maximal production of $O_2^- \bullet$ at much lower concentration (1.25 µM), the maximal production of $O_2^- \bullet$ induced by 6.25 µM $AlCl_3$ was ca. 60 % greater than that induced by $LaCl_3$ (at 1.25 µM) (Figure 1a).

Effects of ROS scavengers and an inhibitor of NADPH oxidase

To clarify the role of the enzymes involved in $AlCl_3$-induced generation of $O_2^- \bullet$, the cell suspension culture was treated with an NADPH oxidase inhibitor, diphenyleneiodonium chloride (DPI, 100 µM), or $O_2^- \bullet$ scavengers, SOD and Tiron, 5 minutes prior to addition of 6.25 µM $AlCl_3$ (Figure 1b). The $AlCl_3$-induced $O_2^- \bullet$ production was effectively inhibited by SOD, Tiron and DPI, indicating that $O_2^- \bullet$ generating activity of NADPH oxidase is involved.

Induction of a transient increase in cytoplasmic Ca^{2+}concentration

Treatment of the tobacco cells with $AlCl_3$ resulted in a rapid and transient increase in $[Ca^{2+}]c$ (Figure 2a). The $O_2^- \bullet$ generation occurred immediately after addition of $AlCl_3$, and the increase in $[Ca^{2+}]c$ was relatively slower, events reaching the maximal level of $[Ca^{2+}]c$ in ca. 1.2 min after addition of $AlCl_3$. Addition of $AlCl_3$ induced the generation of $O_2^- \bullet$ and increase in $[Ca^{2+}]c$ in a dose-dependent manner, however both responses were not optimal at the same $AlCl_3$ concentration (Figure 2b).

Effects of an indole alkaloid from an ectomycorrhizal fungus on the $AlCl_3$-induced ROS production and increase in $[Ca^{2+}]c$

tryptophan betaine, better known as hypaphorine, is an indole alkaloid isolated from an ectomycorrhizal fungus. The $O_2^- \bullet$ generation was sharply reduced by hypaphorine treatments in a dose-dependent manner (Figure 3). Data recorded provide the evidence that the $AlCl_3$-induced increase in $[Ca^{2+}]c$ is not the consequence of $O_2^- \bullet$ production since only the latter responds to hypaphorine treatment.

Conclusion

We propose here that the ROS generated in response to toxic metals may play an important role in the development of metal toxicity including rhizotoxicity. Our present study may provide a novel model for understanding the mechanism of metal toxicity in plants. The ectomycorrhizal fungus *P. tinctorius* is known to accumulate and transfer to the host plant an alkaloid, hypaphorine. We demonstrated *in vitro* that hypaphorine is able to counteract $O_2^- \bullet$ generation in response to $AlCl_3$ treatment. Therefore we propose that such a mechanism, under fungal control, could contribute to increased plant resistance to aluminium toxicity. This new mechanism involving ectomycorrhizal fungi could act in synergy with the well-documented secretion of chelating agents, citric or oxalic acids (cf. evidence in 2nd year report).

Collaboration

The work done for this task by Indian investigator in collaborations with French collaborators in their facility during the visit of Indian PI to Nancy, Champenoux, France.

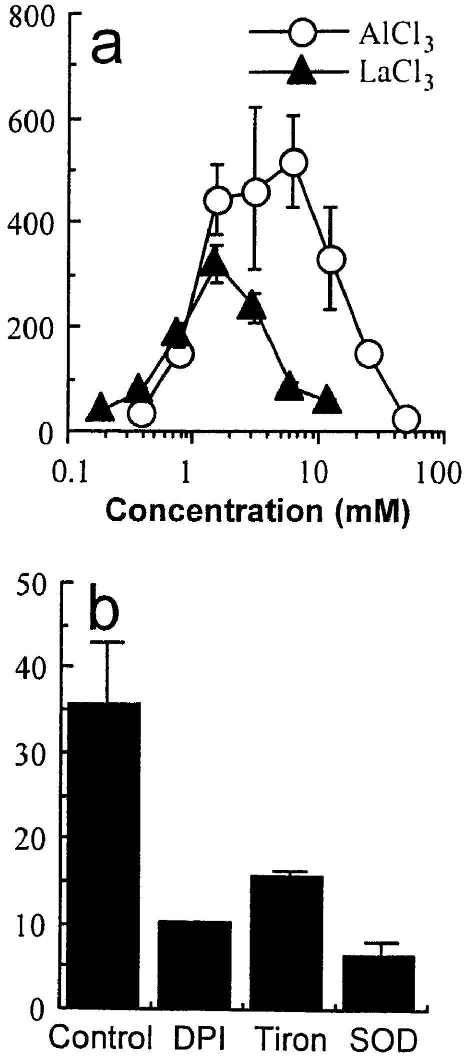
Training at CMR, TERI (11 – 13 June 2002)

Faculty- Dr Valérie Legué, Nancy, France carried out a short duration demonstration exercise based on her expertise.

The following activities were carried out-

a. Observations of living root hairs on the microscope.
b. Visualization of actin cytoskeleton in fixed root hairs before and after the treatment with hypaphorine.
c. Effects of hypaphorine on the Eucalyptus root hairs growth and on their cytoarchitecture.
d. Analysis of papers: published papers were discussed as a group discussion with students.
   i) Cytoplasmic free calcium distribution during the development of root hairs of *Arabidopsis thaliana*.
   ii) *Rhizobium* nod factors induce an increase in sub-apical bundles of actin filaments in *Vicia sativa* root hairs within minutes.
   iii) Abscissic acid signal transduction in the barley aleurone is mediated by phospholipase D activity.
   iv) Involvement of free calcium in actin of cryptogein, a proteinaceous elicitor of hypersensitive reaction in tobacco cells.

Figure 1 Production of $O_2^-\bullet$ by tobacco cell suspension culture. a) Immediate burst of O_2^- production induced by addition of $AlCl_3$ and $LaCl_3$. b) Inhibition of the $AlCl_3$-induced O_2^- production by SOD, Tiron and DPI, indicating that $O_2^-\bullet$ generating activity of NADPH oxidase is involved.



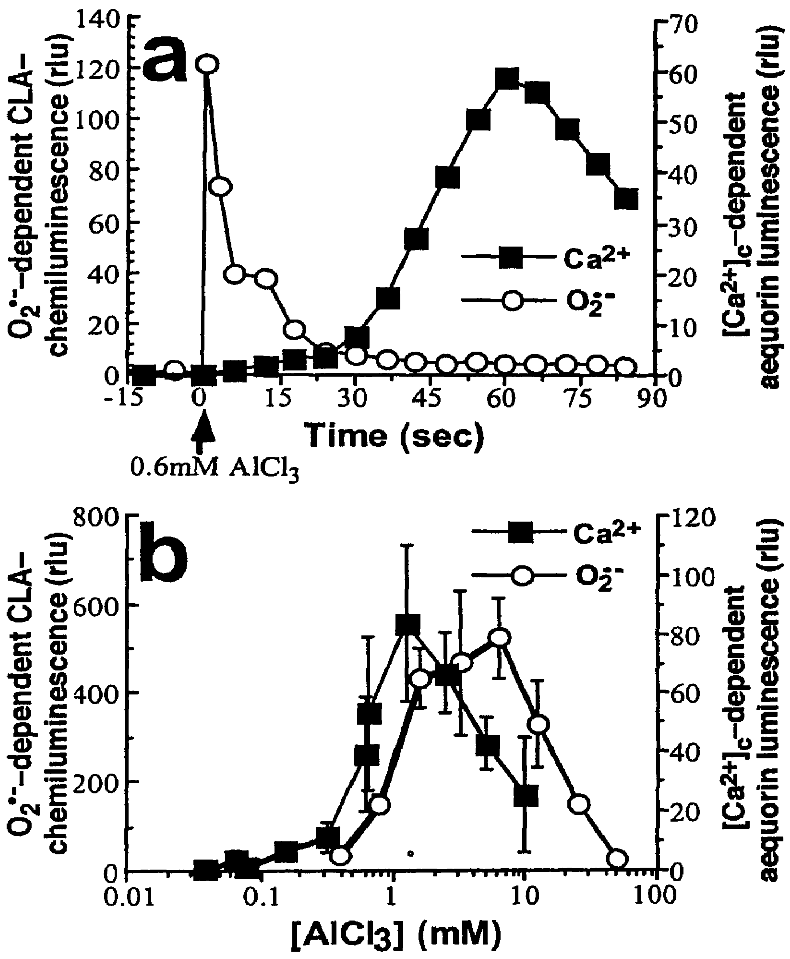
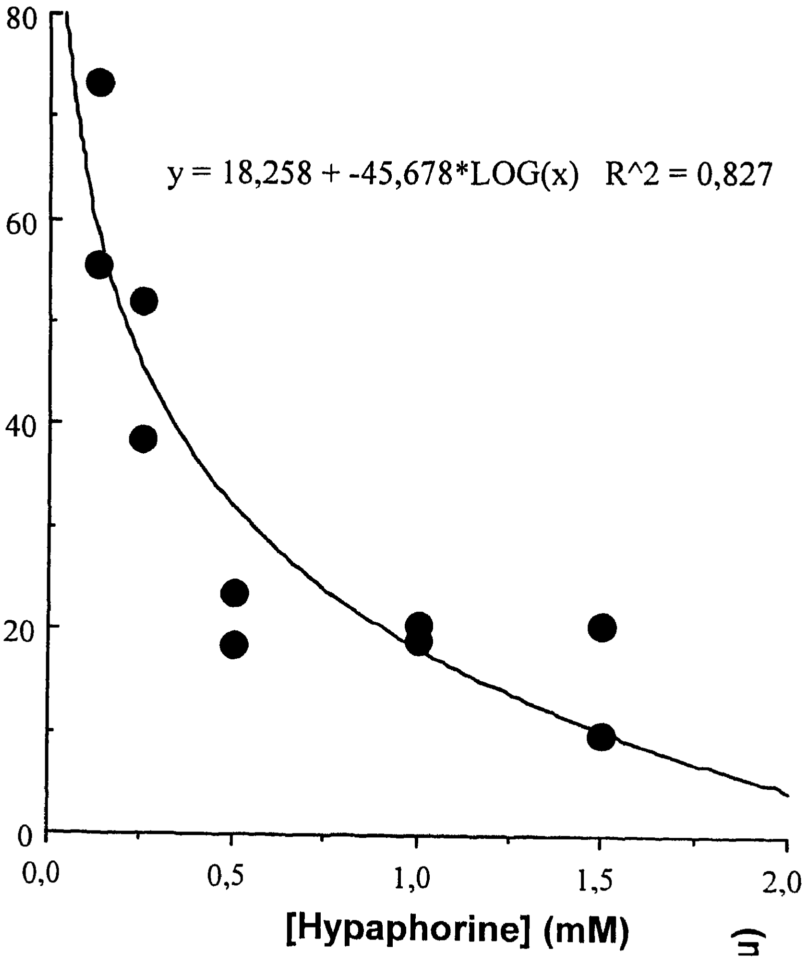


Figure 2: Transient increase in cytoplasmic Ca^{2+}concentration and production of $O_2^{\bullet-}$ by tobacco cells. a) Treatment with $AlCl_3$ resulted in a rapid and transient increase in $[Ca^{2+}]_c$, while the $O_2^{\bullet-}$ generation occurred immediately after addition of $AlCl_3$, the increase in $[Ca^{2+}]_c$ was delayed. b) Addition of $AlCl_3$ induced the generation of $O_2^{\bullet-}$ and increase in $[Ca^{2+}]_c$ in a dose-dependent manner, however both responses were not optimal at the same $AlCl_3$ concentration.

Figure 3 Dose-dependent inhibition by hypaphorine of tobacco cell $O_2^{-}\bullet$ generation induced by $AlCl_3$.



7

Task 6 – Investigation of the root-fungus signalling in an ash pond environment

Nancy - France

(1st year)

In a glasshouse experiment, transgenic clover plants carrying the promoter of the auxin responsive gene, GH3, fused to the reporter gene gusA were inoculated with strains of VAM fungi such as *Gigaspora margarita, G. candida, G. rosea.* Using this model, the regulation of auxin activity in plants during interaction with VAM fungi was investigated. Subsequently the effect of ashes upon plant fungus signalling will be followed. Attempts made to obtain sterile rooted plants of clover were recently shown to yield positive results, which would enable us to study the plant-fungus interaction in sterile conditions, avoiding the interference of other soil micro organisms.

(2nd year)

The experiment described in previous report was abandoned since we were not able to successfully transfer the clover transgenic line to sterile culture conditions. A new hypothesis might be investigated during the third year.

(3rd year)

This task was not investigated further during third year.

8

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9

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Coal ash dependency and subsequent heavy metal uptake by some selected ectomycorrhizal fungi *in vitro*
Communicated

Tiwari P, Reddy U G, and Adholeya A.
Comparative symbiotic events in various coal ash amendments *in vitro* of AM fungi *Glomus intraradices* and Ri T-DNA transformed carrot roots
Communicated

Ray P, Reddy U G, and Adholeya A.
Heavy metal uptake in *Sesbania sesban*, *Mentha arvensis*, and *Vetiver zizanoides* inoculated with three types of VA-mycorrhiza in coal ash
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Investigation of ectomycorrhizal fungal ability to colonise and weather coal ash for ash-pond reclamation
In preparation

Posters presented at conferences

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Comparative symbiotic events of various coal ash amendments *in vitro* on AM fungi *Glomus intraradices* and Ri T-DNA transformed carrot roots
Paper presented at *IMC 7*
Oslo, Norway, 11 – 17 August, 2002

Ray P, Reddy U G, and Adholeya A. 2002
Heavy metal uptake in *Sesbania sesban*, *Mentha arvensis*, and *Vetiver zizanoides* inoculated with three types of VA-mycorrhiza in coal ash
Paper presented at *8th New Phytologist Symposium (NPS 2002)*
Held at the Viikki Biocenter, Helsinki, Finland, 9-14 June 2002

Ray P, Reddy U G, Paul B, Lapeyrie F, and Adholeya A. 2002
Coal ash dependency and subsequent heavy metal uptake by some selected ectomycorrhizal fungi *in vitro*
Paper presented at *IMC 7*
Oslo, Norway, 11 – 17 August 2002

Annexures

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1. Chemical properties of ashes – PA
2. Chemical properties of ashes – ESP
3. a – Coal ash dependency and subsequent heavy metal uptake by some selected ectomycorrhizal fungi *in vitro* (poster)
4. (a, b, c, d, e) – Mass production of ectomycorrhizal fungi *in vitro* and synthesis of *Scleroderma flavidum* and *Laccaria laccata in situ*
4. f - Comparative symbiotic events of various coal ash amendments *in vitro* on AM fungi *Glomus intraradices* and Ri T-DNA transformed carrot roots (poster)
5. a – SEM-EDX analysis of elemental adsorption on the surface of roots and mycelia of AM Fungi
5. b – SEM lab at INRA, Nancy, Champenoux, France
5. (c , d) – Abstract 1 and 2

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ANNEXURE - 1
(WET ASHES)

GRANULOMETRIE 5 FRACTIONS SANS DECARBONATATION.....G/KG

- ARGILE (< 2 MICRONS) : 32
- LIMON FIN (2/20 MICRONS) : 285
- LIMON GROSSIER (20/50 MICRONS) : 384
- SABLE FIN (50/200 MICRONS) : 282
- SABLE GROSSIER (200/2000 MICRONS) : 17

PH EAU .... : 6.9

CALCAIRE (CaCO3) TOTAL..............................G/KG : < 1

PHOSPHORE (P2O5) METHODE DUCHAUFOUR.....................G/KG : 0.237

PHOSPHORE (P2O5) METHODE OLSEN..........................G/KG : 0.037

CAPACITE D'ECHANGE CATIONIQUE METHODE METSON...CMOL+/KG : 0.4

CALCIUM (Ca) ECHANGEABLE ACETATE D'AMMONIUM....CMOL+/KG : 0.[illegible]

SODIUM (Na) ECHANGEABLE ACETATE D'AMMONIUM.....CMOL+/KG : 0.012

MANGANESE (Mn) ECHANGEABLE ACETATE D'AMMONIUM.....MG/KG : 0.7

MAGNESIUM (Mg) ECHANGEABLE ACETATE D'AMMONIUM..CMOL+/KG : 0.02

POTASSIUM (K) ECHANGEABLE ACETATE D'AMMONIUM...CMOL+/KG :

CARBONE ET AZOTE SELON NF [illegible] ET [illegible] ....G/KG

- CARBONE ORGANIQUE..G : 5.50
- AZOTE ORGANIQUE..G : 0.12

ALUMINIUM (Al) ECHANGEABLE PAR KCL 1.0 MOL/L...CMOL+/KG : < 0.05

BORE (B) SOLUBLE A L'EAU BOUILLANTE.................MG/KG : 0.08

CALCIUM (Ca) TOTAL HF............................G/100 G : 0.21

MAGNESIUM (Mg) TOTAL HF..........................G/100 G : 0.19

POTASSIUM (K) TOTAL HF...........................G/100 G : 0.68

SODIUM (Na) TOTAL HF.............................G/100 G : 0.03

CUIVRE (Cu) TOTAL HF..................................MG/KG : 57.6

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RESULTATS

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PAGE 2

FER (Fe) TOTAL HF	G/100 G :	1.8
MANGANESE (Mn) TOTAL HF	MG/KG :	154.7
ZINC (Zn) TOTAL HF	MG/KG :	90.1
PHOSPHORE (P2O5) TOTAL HF	G/100 G :	0.173
CHROME (Cr) TOTAL HF	MG/KG :	134.6
ALUMINIUM (Al) TOTAL HF	G/100G :	13.30
NICKEL (Ni) TOTAL HF	MG/KG :	50.8
COBALT (Co) TOTAL HF	MG/KG :	17.0
BARYUM (Ba) TOTAL HF	MG/KG :	[illegible]
LITHIUM (Li) TOTAL HF	MG/KG :	[illegible]
PLOMB (Pb) TOTAL HF	MG/KG :	40.[illegible]
CADMIUM (Cd) TOTAL HF	MG/KG :	0.[illegible]
STRONTIUM (Sr) TOTAL HF	MG/KG :	[illegible]
MOLYBDENE (Mo)	MG/KG :	1.[illegible]5
VANADIUM (V) TOTAL HF	MG/KG :	155.[illegible]
THALLIUM (Tl) TOTAL HF	MICROGRAMME/KG :	736
AZOTE NITRIQUE (N DE NO3)	MG/KG :	Echantill.
AZOTE AMMONIACAL (N DE NH4)	MG/KG :	Echantill.
CUIVRE (Cu) EXTRACTIBLE A L'EDTA	MG/KG :	0.4
MANGANESE (Mn) EXTRACTIBLE A L'EDTA	MG/KG :	0.6
ZINC (Zn) EXTRACTIBLE A L'EDTA	MG/KG :	0.7
FER (Fe) EXTRACTIBLE A L'EDTA	MG/KG :	6.9

ANNEXURE - 2
(DRY ASHES)

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RESULTATS

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LE 15/09/00
PAGE 1

ANNEXE 2 : DRY ASHES

GRANULOMETRIE 5 FRACTIONS SANS DECARBONATATION	G/KG	
- ARGILE (< 2 MICRONS)		47
- LIMON FIN (2/20 MICRONS)		447
- LIMON GROSSIER (20/50 MICRONS)		301
- SABLE FIN (50/200 MICRONS)		193
- SABLE GROSSIER (200/2000 MICRONS)		12
PH EAU		5.3
CALCAIRE (CaCO3) TOTAL	G/KG	< 1
PHOSPHORE (P2O5) METHODE DUCHAUFOUR	G/KG	0.424
PHOSPHORE (P2O5) METHODE OLSEN	G/KG	0.036
CAPACITE D'ECHANGE CATIONIQUE METHODE METSON	CMOL+/KG	0.4
CALCIUM (Ca) ECHANGEABLE ACETATE D'AMMONIUM	CMOL+/KG	0.7
SODIUM (Na) ECHANGEABLE ACETATE D'AMMONIUM	CMOL+/KG	0.024
MANGANESE (Mn) ECHANGEABLE ACETATE D'AMMONIUM	MG/KG	Echantill.Insuff.
MAGNESIUM (Mg) ECHANGEABLE ACETATE D'AMMONIUM	CMOL+/KG	0.12
POTASSIUM (K) ECHANGEABLE ACETATE D'AMMONIUM	CMOL+/KG	0.070
CARBONE ET AZOTE SELON NF 10694 ET 13878	G/KG	
- CARBONE ORGANIQUE	G/KG	4.19
- MATIERES ORGANIQUES	G/KG	7.2
- AZOTE ORGANIQUE	G/KG	0.04
RAPPORT CARBONE ORGANIQUE SUR AZOTE TOTAL		104.75
ALUMINIUM (Al) ECHANGEABLE PAR KCL 1.0 MOL/L	CMOL+/KG	0.20
BORE (B) SOLUBLE A L'EAU BOUILLANTE	MG/KG	0.46
CALCIUM (Ca) TOTAL HF	G/100 G	0.22
MAGNESIUM (Mg) TOTAL HF	G/100 G	0.20
POTASSIUM (K) TOTAL HF	G/100 G	0.71
SODIUM (Na) TOTAL HF	G/100 G	0.03

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RESULTATS

ECHANTILLON: 6-118-1- 2/17571

LE 15/09/00

CUIVRE (Cu) TOTAL HF	MG/KG :	67.5
FER (Fe) TOTAL HF	G/100 G :	1.85
MANGANESE (Mn) TOTAL HF	MG/KG :	168.3
ZINC (Zn) TOTAL HF	MG/KG :	135.0
PHOSPHORE (P2O5) TOTAL HF	G/100 G :	0.295
CHROME (Cr) TOTAL HF	MG/KG :	144.8
ALUMINIUM (Al) TOTAL HF	G/100G :	13.24
NICKEL (Ni) TOTAL HF	MG/KG :	58.0
COBALT (Co) TOTAL HF	MG/KG :	23.3
BARYUM (Ba) TOTAL HF	MG/KG :	394.50
LITHIUM (Li) TOTAL HF	MG/KG :	43.96
PLOMB (Pb) TOTAL HF	MG/KG :	61.1
CADMIUM (Cd) TOTAL HF	MG/KG :	0.480
STRONTIUM (Sr) TOTAL HF	MG/KG :	181.2
SILICIUM (Si) TOTAL PAR FLUORESCENCE X	G/100 G :	30.29
ARSENIC (As) TOTAL	MG/KG :	5.56
MOLYBDENE (Mo)	MG/KG :	6.70
VANADIUM (V) TOTAL HF	MG/KG :	170.3
THALLIUM (Tl) TOTAL HF	MICROGRAMME/KG :	1348
AZOTE NITRIQUE (N DE NO3)	MG/KG :	4.20
AZOTE AMMONIACAL (N DE NH4)	MG/KG :	0.20
CUIVRE (Cu) EXTRACTIBLE A L'EDTA	MG/KG :	0.7
MANGANESE (Mn) EXTRACTIBLE A L'EDTA	MG/KG :	1.7

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RESULTATS

ECHANTILLON: 6-118-1- 2/17571

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PAGE 3

ZINC (Zn) EXTRACTIBLE A L'EDTA	MG/KG :	1.3
FER (Fe) EXTRACTIBLE A L'EDTA	MG/KG :	15.6

ANNEXURE - 3a

Coal ash dependency and subsequent heavy metal uptake by some selected ectomycorrhizal fungi *in vitro*

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[2]Laboratoire des sciences de la Vigne, Institut Jules Guyot, Universite de Bourgogne, P.O. Box 138, 21004 Dijon, France
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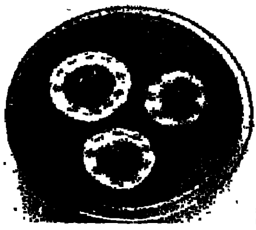
Coal based thermal power stations generate over 70% of the total electric power in India, using low grade coal with a high ash content (40%–50%) leading to the production of about 90 million tones of ash/year Besides requiring large tracts of land for ash disposal (65000 acres of land nationwide), air pollution results in the form of fugitive dust. There is need for an effective method of preventing ash particles from getting re suspended in the air *This can be achieved by developing green cover extensively on and around disposal* sites which will further prevent contamination of ground water and soil otherwise caused by the down ward movement of metals. Inoculations of forest tree seedlings with ecologically adapted ectomycorrhizal fungi could be the best option for the future.

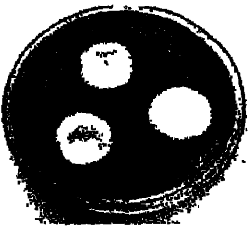
In this study, efforts were made to look at the influence of ashes containing many heavy metals. *Therefore the ashes were moistened with synthetic media to assess the ash* dependency of the ectomycorrhizal isolates and extent of the heavy metal accumulated in the mycelia grown *in vitro* We selected six metals namely Al, Ni, Pb, Cr, Cd, Fe based on their relative abundance in the ash and their potential toxicity. This toxicity is related to their bio availability, which is correlated with pH, which was monitored in this work.

The isolates tested, namely *Laccaria fraterna* (EM-1083); *Pisolithus tinctorius* (EM-1081), *Scleroderma cepa* (EM-1233); *Pisolithus tinctorius* (EM-1290); *Scleroderma verucosum* (EM-1283), *Pisolithus tinctorius* (EM-1293) were obtained from the Centre for Mycorrhizal Culture Collection, Tata Energy Research Institute, New Delhi, India.



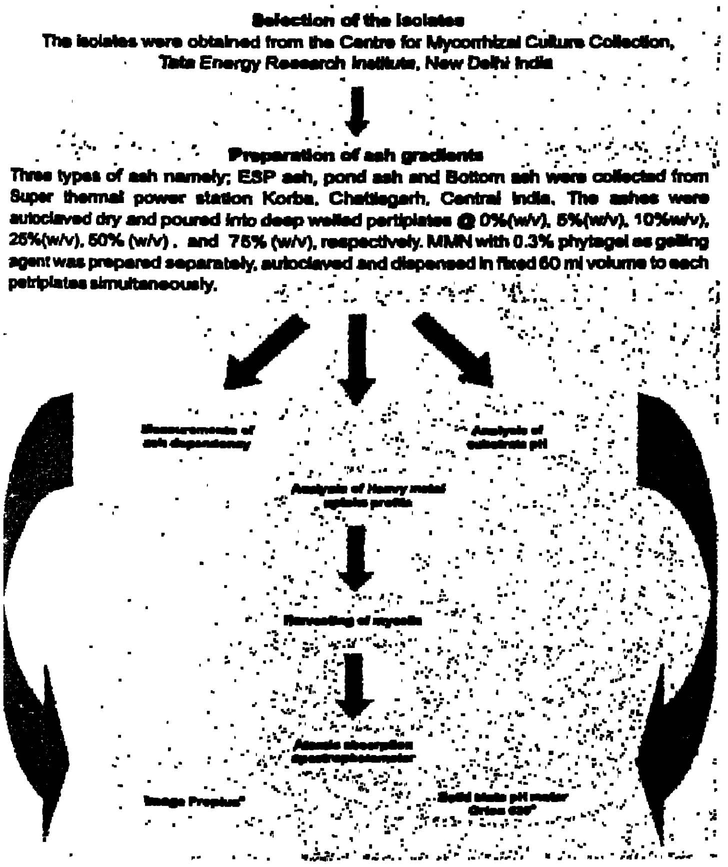
Scleroderma verrucosum (EM-1283) growing on Coal ash

Aggregation of ash particles by fungal mycelia



Scleroderma cepa (EM-1233) growing on Coal ash

Materials and methods



Selection of the isolates

The isolates were obtained from the Centre for Mycorrhizal Culture Collection, *Tata Energy Research Institute, New Delhi India*

Preparation of ash gradients

Three types of ash namely; ESP ash, pond ash and Bottom ash were collected from Super thermal power station Korba, Chattisgarh, Central India. The ashes were autoclaved dry and poured into deep welled pertiplates @ 0%(w/v), 5%(w/v), 10%w/v), 25%(w/v), 50% (w/v) . and 75% (w/v), respectively. MMN with 0.3% phytagel as gelling agent was prepared separately, autoclaved and dispensed in fixed 60 ml volume to each petriplates simultaneously.

Measurements of ash dependency

Analysis of substrate pH

Analysis of Heavy metal uptake profile

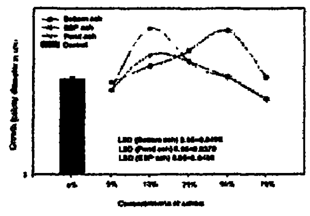
Harvesting of mycelia

Atomic absorption spectrophotometer

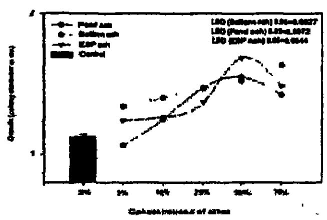
Image Proplus®

Solid state pH meter Orion 420®

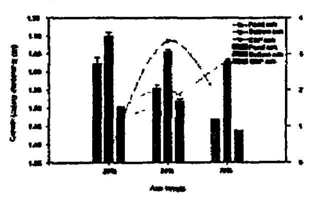
Growth response of *Laccaria fraterna* (EM-1083) at different concentrations of three types of ashes



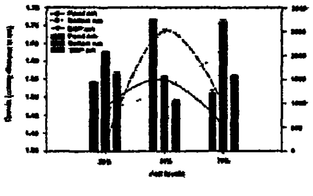
Growth response of *Pisolithus tinctorius* (EM-1290) at different concentrations of three types of ashes



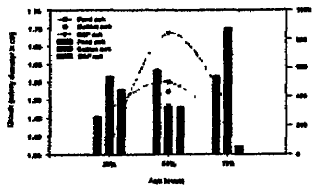
Growth response and corresponding Cadmium uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)



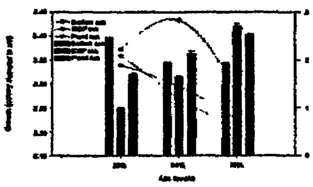
Growth response and corresponding Aluminium uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)

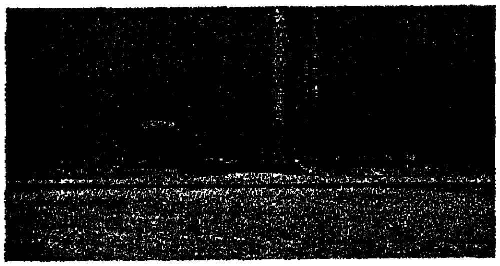


Growth response and corresponding Iron uptake in the mycelia of *Pisolithus tinctorius* (EM-1290)



Growth response and corresponding Chromium uptake in the mycelia of *Laccaria fraterna* (EM-1083)





Ash pond, Super Thermal Power Station, Korba

Salient findings

- Colony morphology varied greatly in the ash amended media.
- Substrate pH has shifted towards the acidic range due to release of metabolites like oxalic acids by the *ectomycorrhizal fungi*.
- Coal ash acted as the buffering agent which nullifies the effect of the acids secreted by the ectomycorrhizal fungi.
- ESP ash promoted the growth of ectomycorrhiza in low and moderate ash amended levels
- *Pisolithus tinctorius* (EM-1290) is tolerant to most of the metals among the six isolates tested.
- This isolate, in association with Eucalyptus could be used for the reclamation purpose in coal ash over burdened sites.

Acknowledgements

The present study was supported by funding from Indo French Centre for the Promotion of Advanced Research (IFCPAR), Project No. 2109-1.

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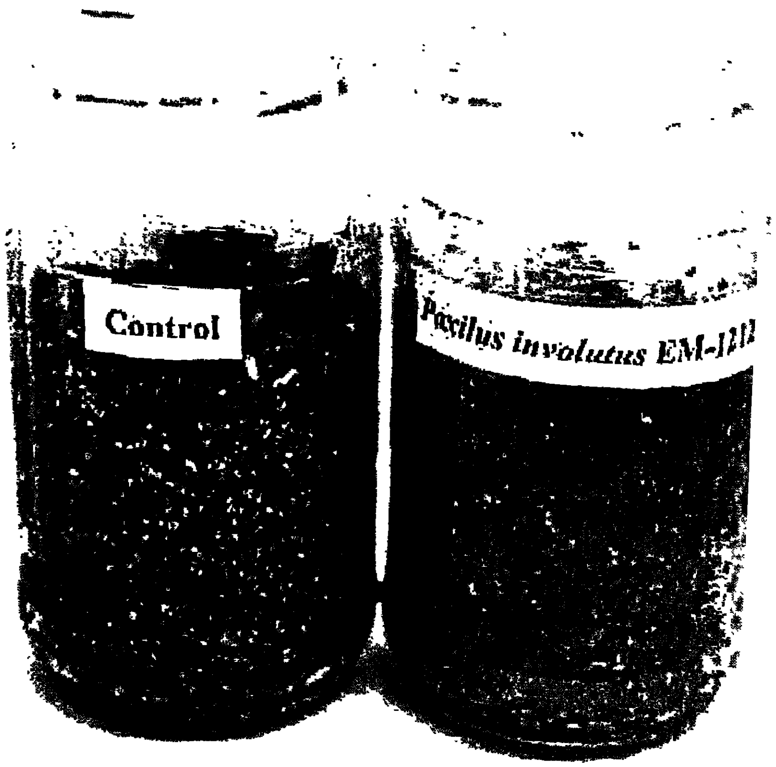
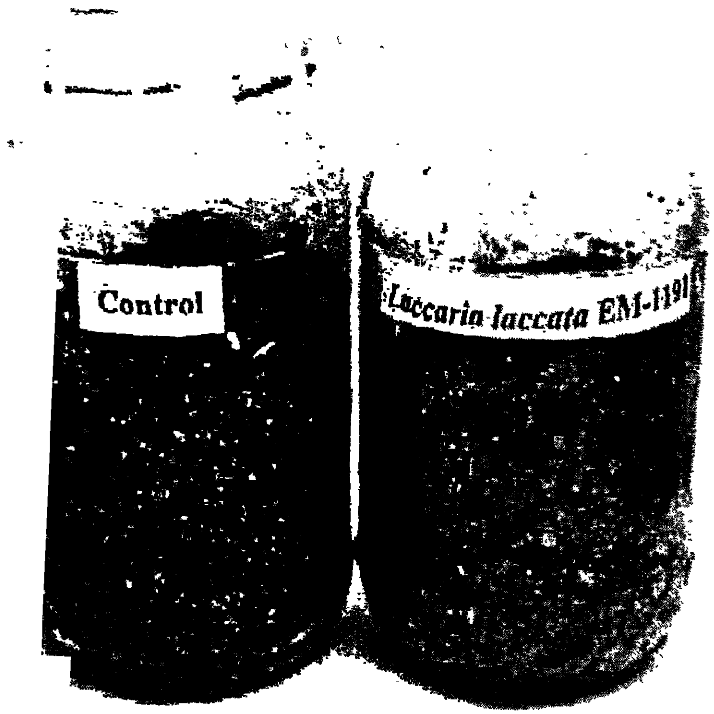
Lapeyrie, F., Ranger, J. and Vairelles, D. 1991. Phosphate-solubilizing activity of the ectomycorrhizal fungi invitro. Can .J.Bot.69:342–346.

Mass production of ectomycorrhizal fungi *in vitro*





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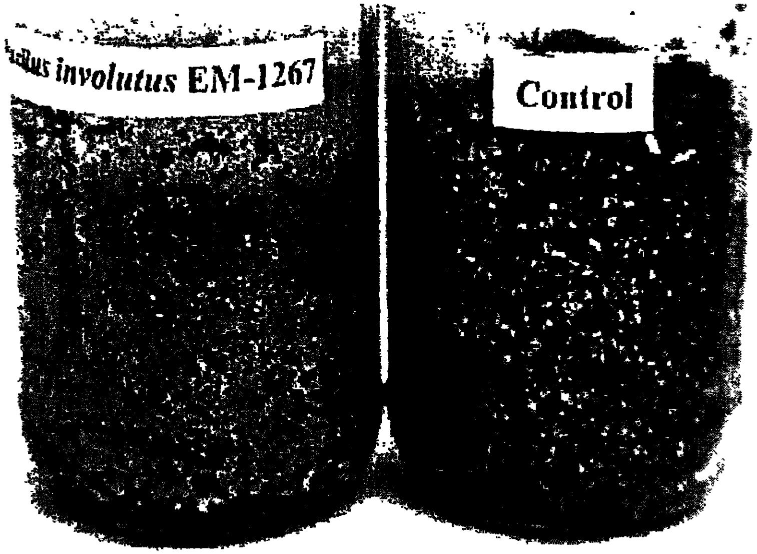
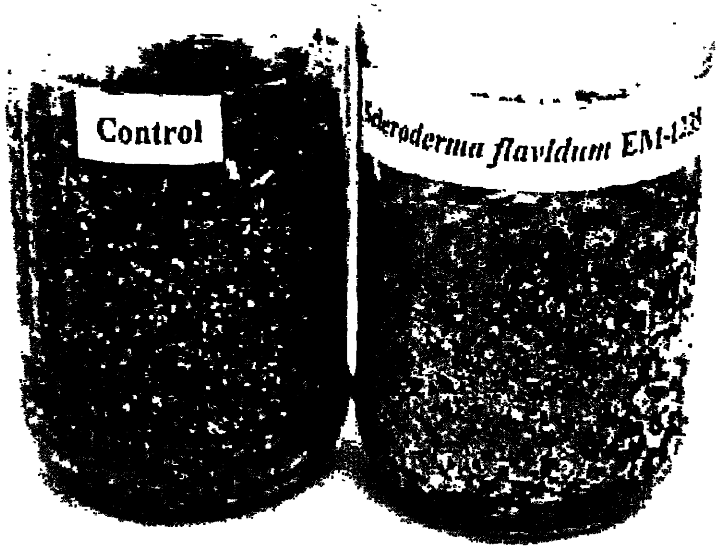
Annexure 4 b

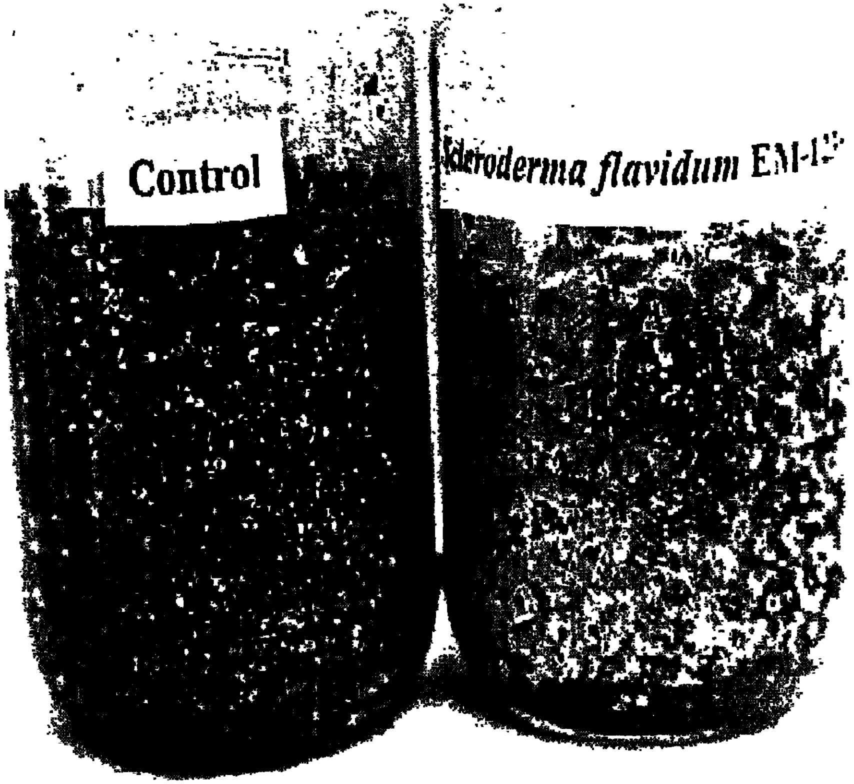
Mass production of ectomycorrhizal fungi *in vitro*

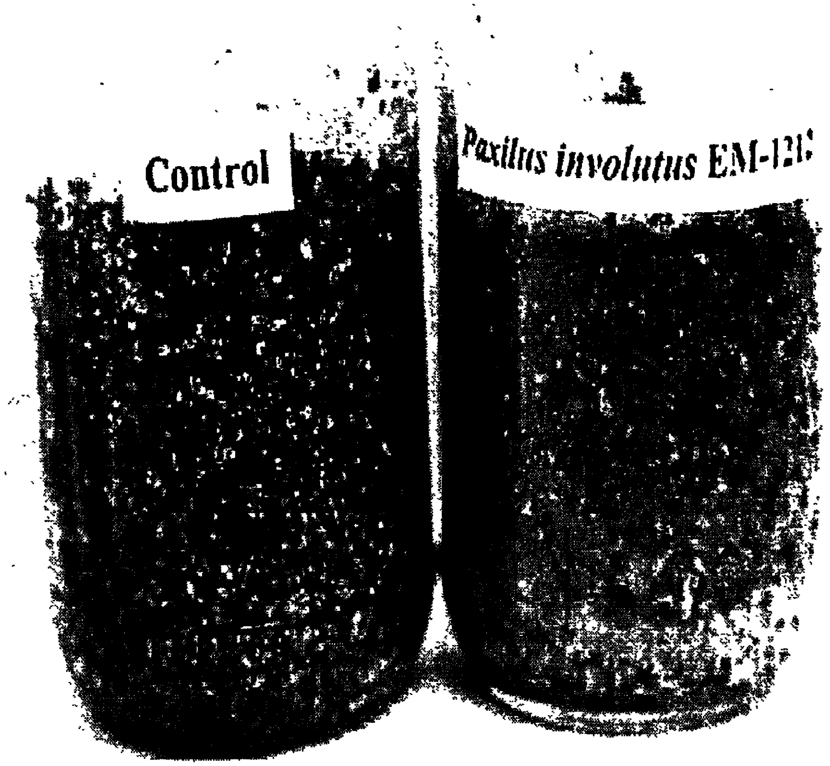




Annexure 4 c

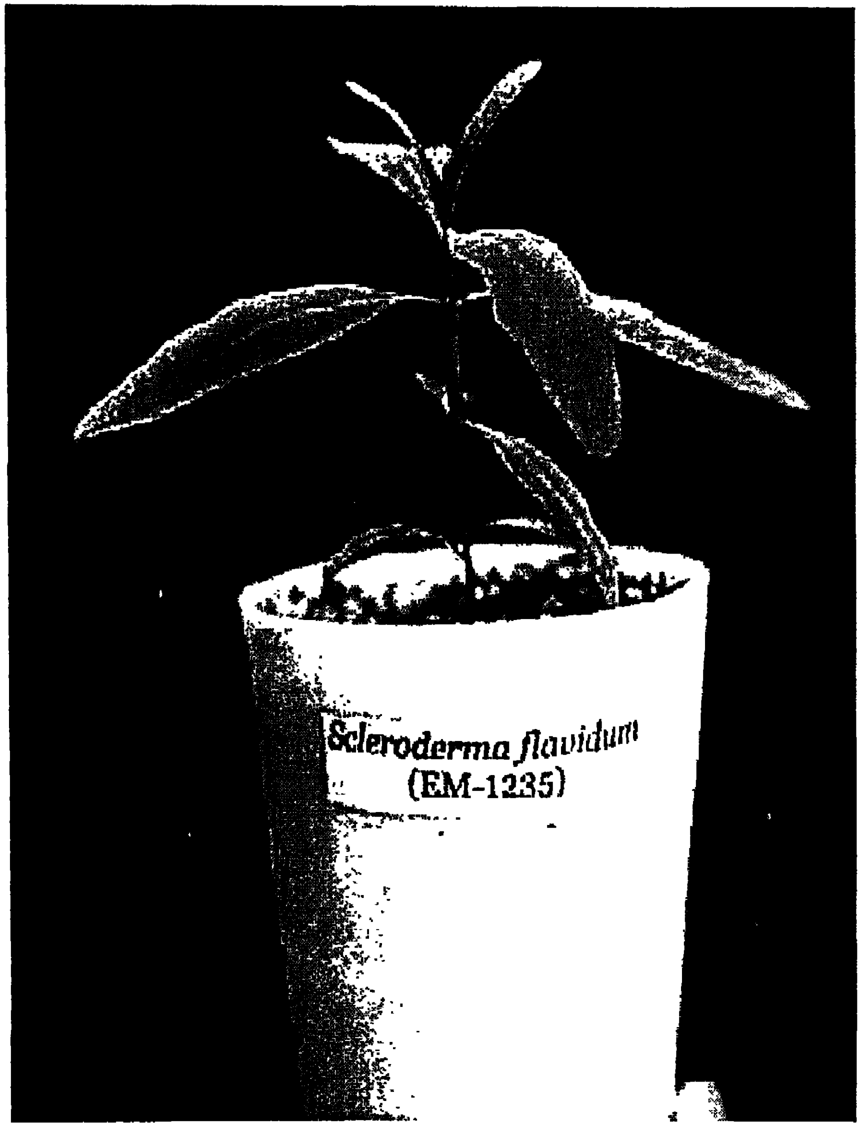
Mass production of ectomycorrhizal fungi *in vitro*

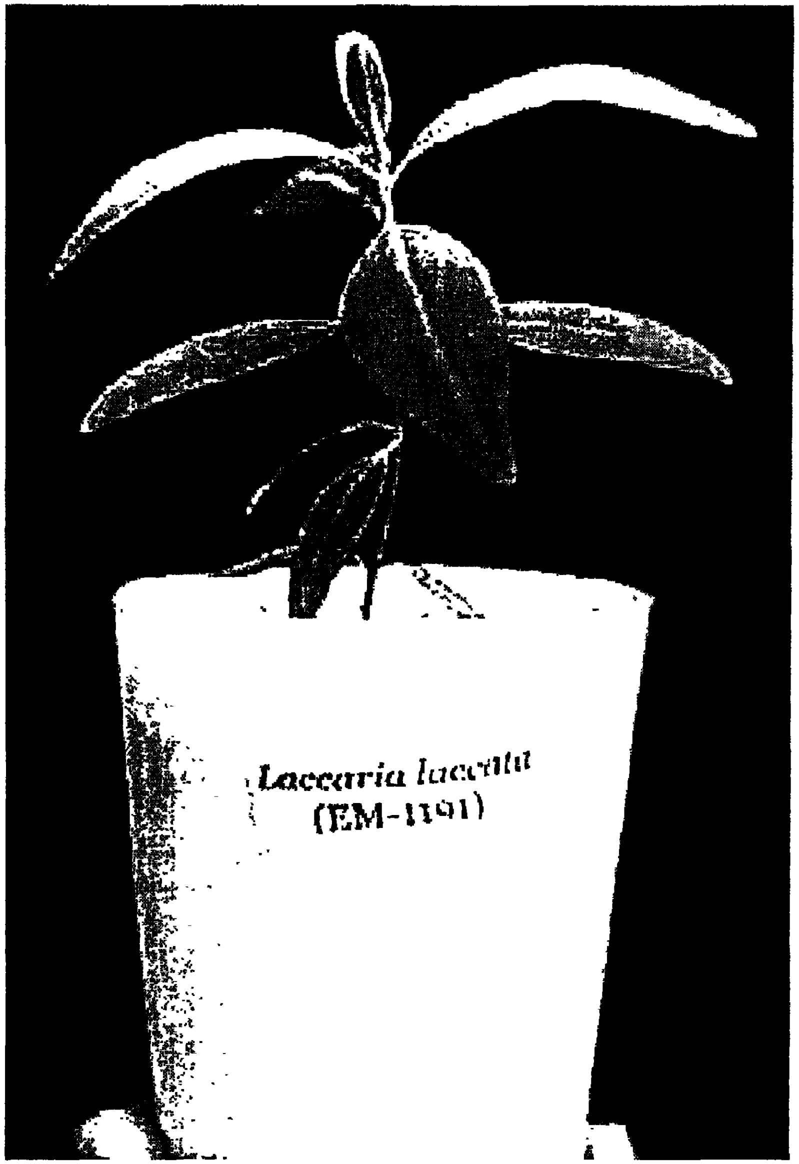




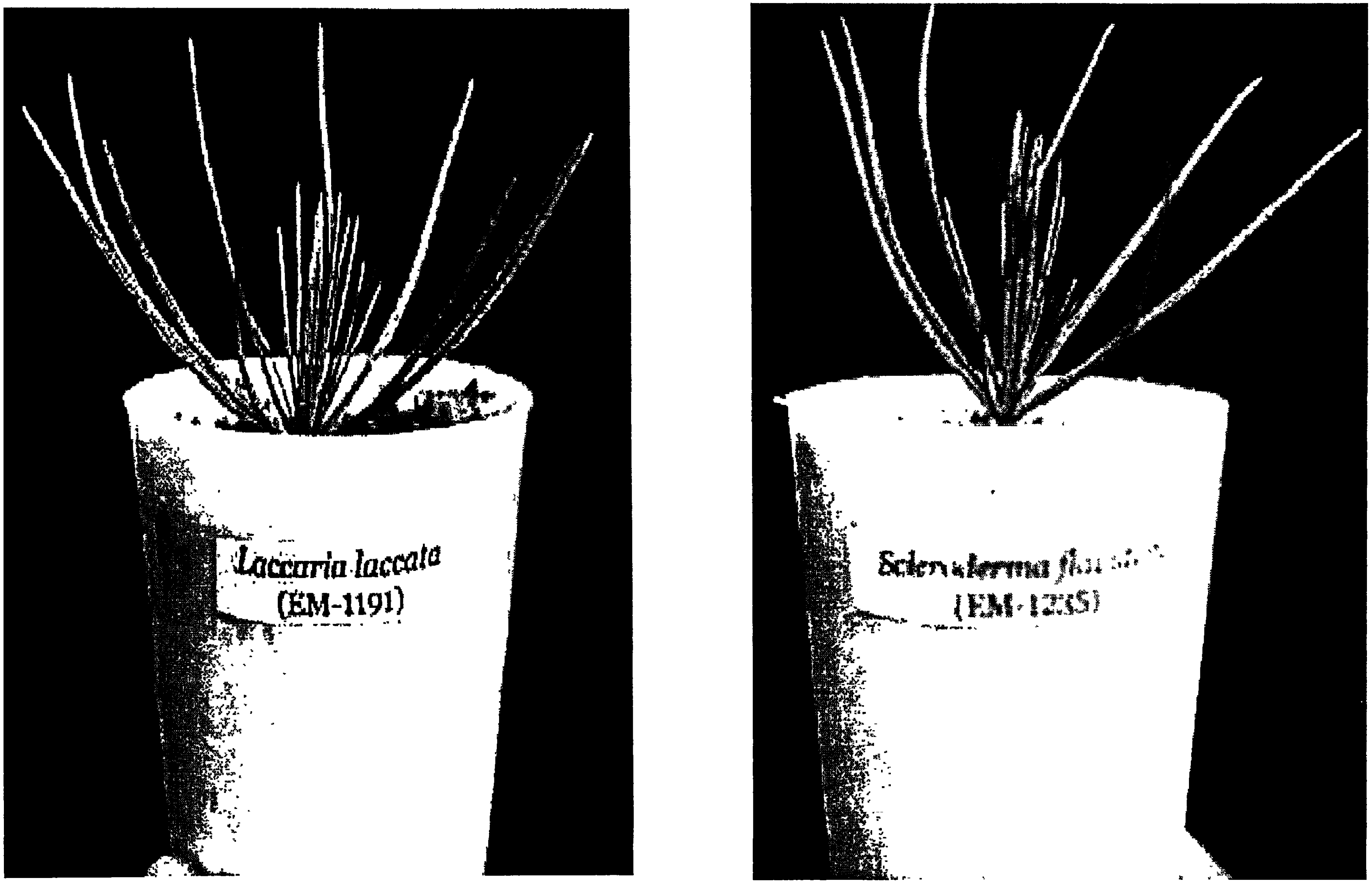
Annexure 4 d

Synthesis of *Scleroderma flavidum* & *Laccaria laccata in situ*





Synthesis of *Scleroderma flavidum* & *Laccaria laccata in situ*



ANNEXURE - 45

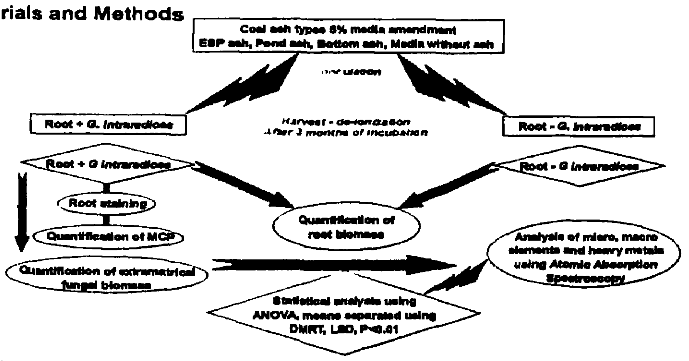
Comparative symbiotic events of various coal ash amendments *in vitro* on AM fungi *Glomus intraradices* and Ri T-DNA transformed carrot roots

Pragati Tiwari, U Gangi Reddy and Alok Adholeya
Tata Energy Research Institute, Habitat place, Darbari Seth Block, Lodhi Road, New Delhi – 110 003, India

Introduction

Elevated concentrations of heavy metals and trace elements are major environmental problems of contaminated soils and large wasteland dumps generated from coal ash from thermal power plants. Such areas are totally devoid of vegetation and pose a long-term risk to the quality of the *environment and sustainable food production. The rehabilitation of such disturbed ecosystems is* a major challenge Reclamation through biological means is most desirable under the present scenario of excessive fertilization and increasing chemical inputs. In India, approximately 30,000 acres of wasteland is being generated by thermal power plants, producing approximately 90 million tonnes of coal ash annually In the present investigation, the role of fungi as biological *barriers against the transfer of excessive elements to the plant shoots* has been studied *in vitro*. Three different types of ash i.e , ESP ash, pond ash and bottom ash obtained from the Korba Super Thermal Power Station located in Chattisgarh, Central India were used to amend media A comprehensive elemental analysis was conducted to evaluate the absorption, translocation and tolerance of macro and microelements and heavy metals in the extramatrical mycelial biomass. The difference in the mycorrhizal expression when exposed to different types of ashes with different physico-chemical properties was observed clearly indicating the higher accumulation of certain elements leading to the poor responsiveness to various symbiotic events of mycorrhizal spread. These elements were not translocated to roots thereby indicating a positive role to AM fungi towards plant protection and environmental cleaning. This is the first report of direct ash *amendment with multiple heavy metal accumulation in AM fungal biomass in vitro.*

Materials and Methods



Coal ash types 5% media amendment
ESP ash, Pond ash, Bottom ash, Media without ash

...ulation

Root + *G. intraradices*

Harvest - de-ionization
After 3 months of incubation

Root - *G. intraradices*

Root + *G intraradices*

Root staining

Quantification of MCP

Quantification of extramatrical fungal biomass

Quantification of root biomass

Root - *G intraradices*

Analysis of micro, macro elements and heavy metals *using Atomic Absorption Spectroscopy*

Statistical analysis using ANOVA, means separated using DMRT, LSD, P<0.01

Results

Figure 1 Comparison of root length profile of roots grown in different coal ash amendments

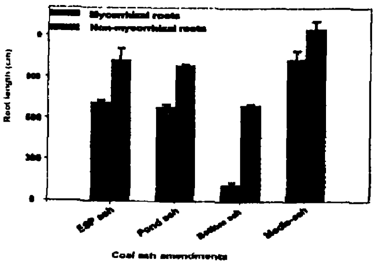


Figure 2 Mycorrhizia colonization percentage (MCP) in roots grown in different coal ash amended media

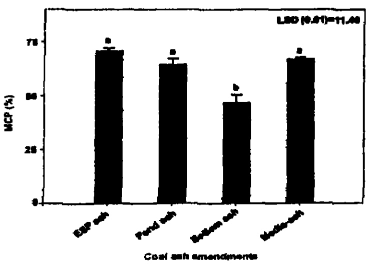


Figure 3 Comparison of extramatrical sporulation in different coal ash amended media

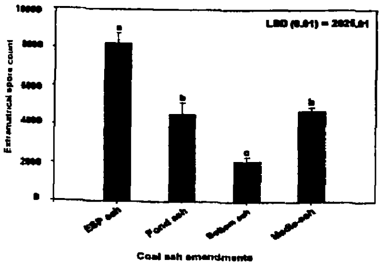


Figure 4 Comparison of extramatrical mycelial spread in different coal ash amended media

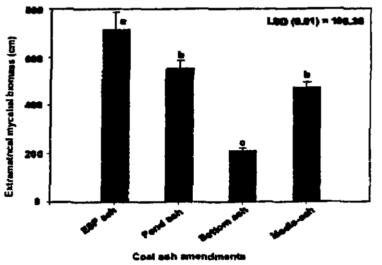
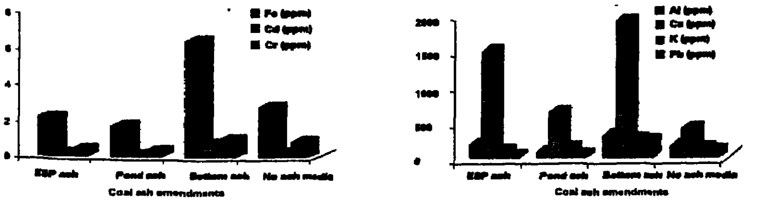


Figure 5 and 6 Micro, macro elemental and heavy metal absorption in extramatrical AM fungal biomass grown in different ash amendments



G intraradices with transformed carrot roots extramatrical showing extensive fungal spread with sporulation inside the media

Table 1 Physio-chemical properties of ashes used in media amendments

Physico- chemical properties		ESP ash	Pond ash	Bottom ash
Particle size distribution gm/Kg	***< 2 microns***	47	32	57
	2/20 microns	447	285	317
	20/ 50 microns	301	384	351
	50/ 200 microns	193	282	275
	200/ 2000 microns	12	17	0
	pH	5.3	6.9	9.3
Cation exchange capacity (CEC)		0.4	0.4	1.0
Cmolc /Kg of ash				
Electrical conductivity (EC)		0.48	0.2	0.28
The values of elements are in ppm				
Zinc (Zn) Total HF		135.0	90.1	50.3
Iron (Fe) Total HF		1850	1810	3830
Manganese (Mn) Total HF		168.3	154.7	398.7
Copper (Cu) Total HF		67.5	57.6	48.3
Lead (Pb) Total HF		61.1	40.3	16.9
Nickel (Ni) Total HF		58.0	50.8	39.5
Cobalt (Co) Total HF		23.3	17.0	85.1
Cadmium (Cd) Total HF		0.48	0.26	0.07
Chromium (Cr) Total HF		144.8	134.6	114.7
Sodium (Na) Total HF		30	30	30
Aluminum (Al) Total HF		13240	13300	11830
Magnesium (Mg) Total HF		200	190	220
Calcium (Ca) Total HF		220	210	360
Potassium (K) Total HF		710	680	630
Molybdenum (Mo) Total HF		6.70	1.65	1.50
Arsenic (As) Total HF		5.56	BDL	< 0.50

Intraradical spread of *G intraradices* showing numerous vesicles formation

Acknowledgements

The authors wish to acknowledge Indo–French Centre for Promotion of Advanced Research (IFCPAR) *for the financial assistance of the* project (IFC 2109-1) for the present study.

Salient findings

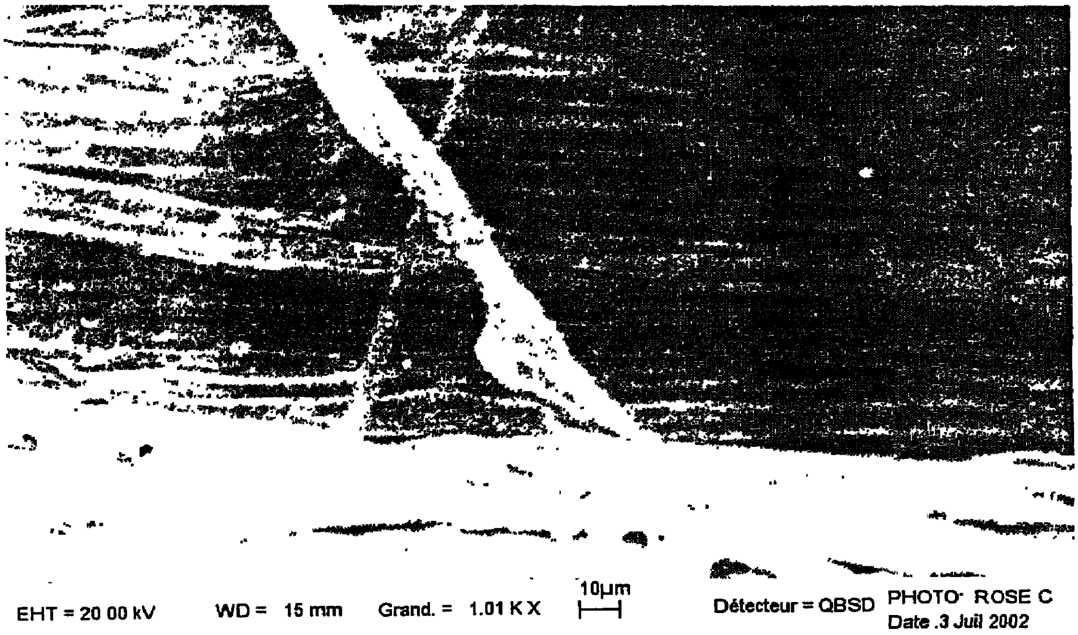
- Root biomass measured as root length was higher in non-mycorrhizal roots than mycorrhizal roots in all treatments irrespective of ash amendments (Figure 1). This is a constant feature under *in vitro* conditions as both symbionts compete for the carbon from the media it was least in bottom ash treatment.
- Mycorrhizal colonization (Figure 2) was highest in ESP ash followed by media without ash and pond ash, it was least expressed in bottom ash treatment.
- Extramatrical mycorrhizal biomass and spore count also followed a similar pattern (Figures 3 & 4), and were highest in ESP ash followed by pond ash and media without ash, again least in bottom *ash treatment.*
- The enhanced accumulation of these micro, macro and heavy metals including known beneficial and debatable stressful elements in extramatrical mycelial biomass positively influence the extent of mycorrhization and fungal spread.
- These elements like Fe, Cd, Cr, Pb, Al, Ca and K were very high in bottom ash amended *extramatrical mycelial* biomass (fungal immobilization) which appear to have led to the failure of mycorrhizal machinery to function normally as in other treatments (Figure 5 and 6).

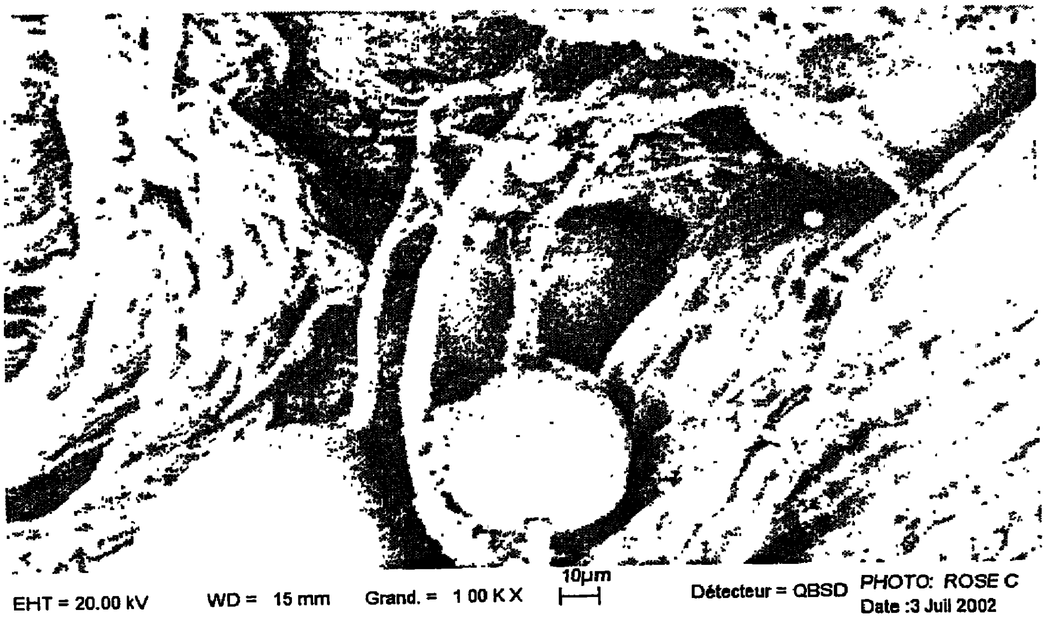
Conclusions

- It is difficult to point on a single element for such a stressful expression of same mycorrhizal isolate in bottom ash treatment as compared to other treatments. *This expression seems to* be a cumulative effect of many elements and heavy metals.
- *The findings once again draw our attention* to the fungal adaptations occurring in nature that enhance such selective filtering of stress inducing elements. These may be important mechanisms responsible for holding them to entry in the food chain, highlighting the demand of in depth study in future for judicious utilization of mycorrhizal potential for land reclamation through biological means.

Annexure – 5a

SEM – EDX analysis of elemental adsorption on the surface of roots and mycelia of AM Fungi





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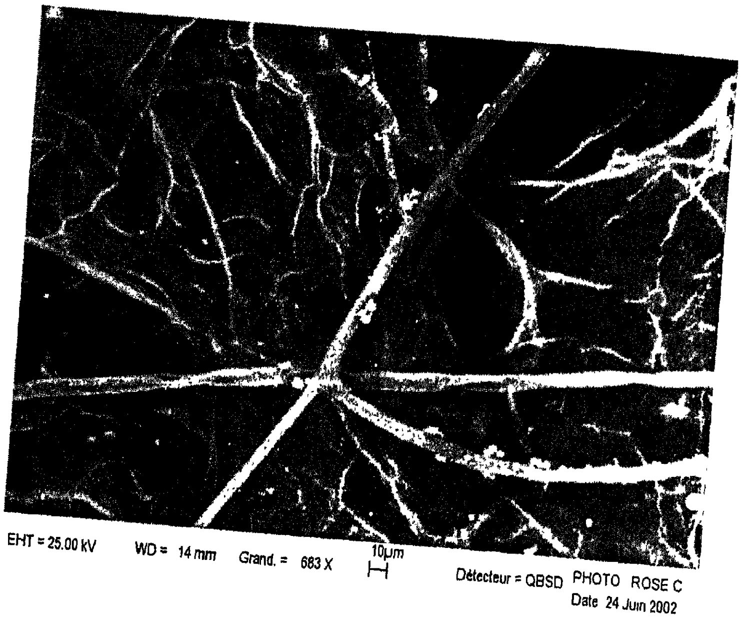
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WD = 14 mm
Grand. = 683 X
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Détecteur = QBSD
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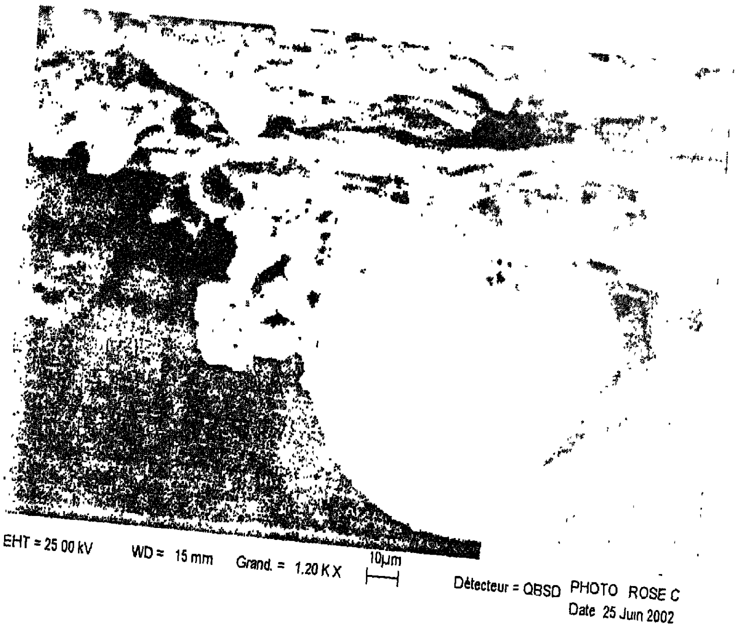
Date 24 Juin 2002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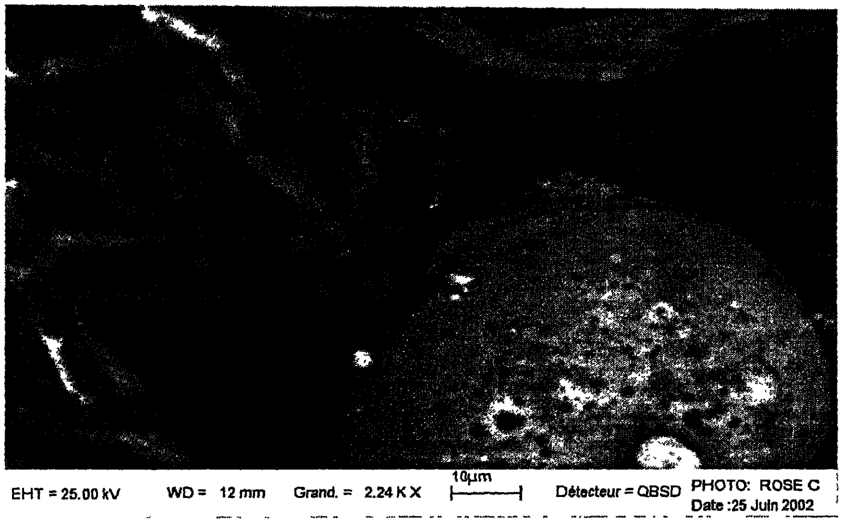
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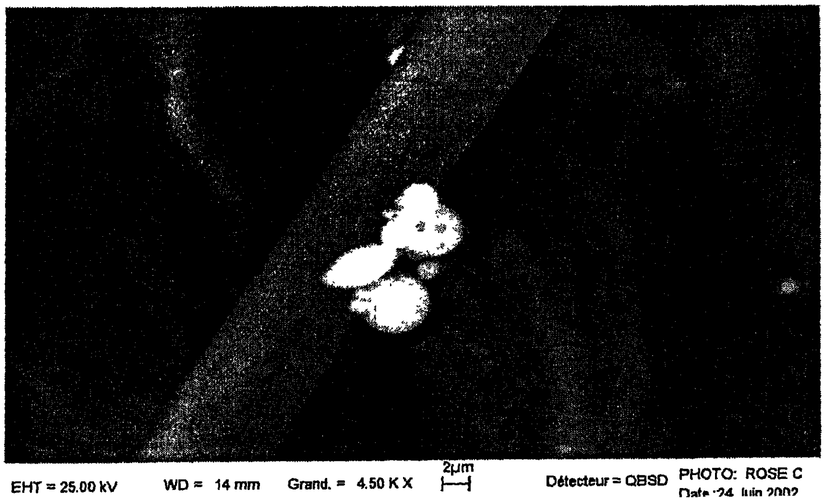
EHT = 25.00 kV
WD = 14 mm
Grand. = 4.50 K X
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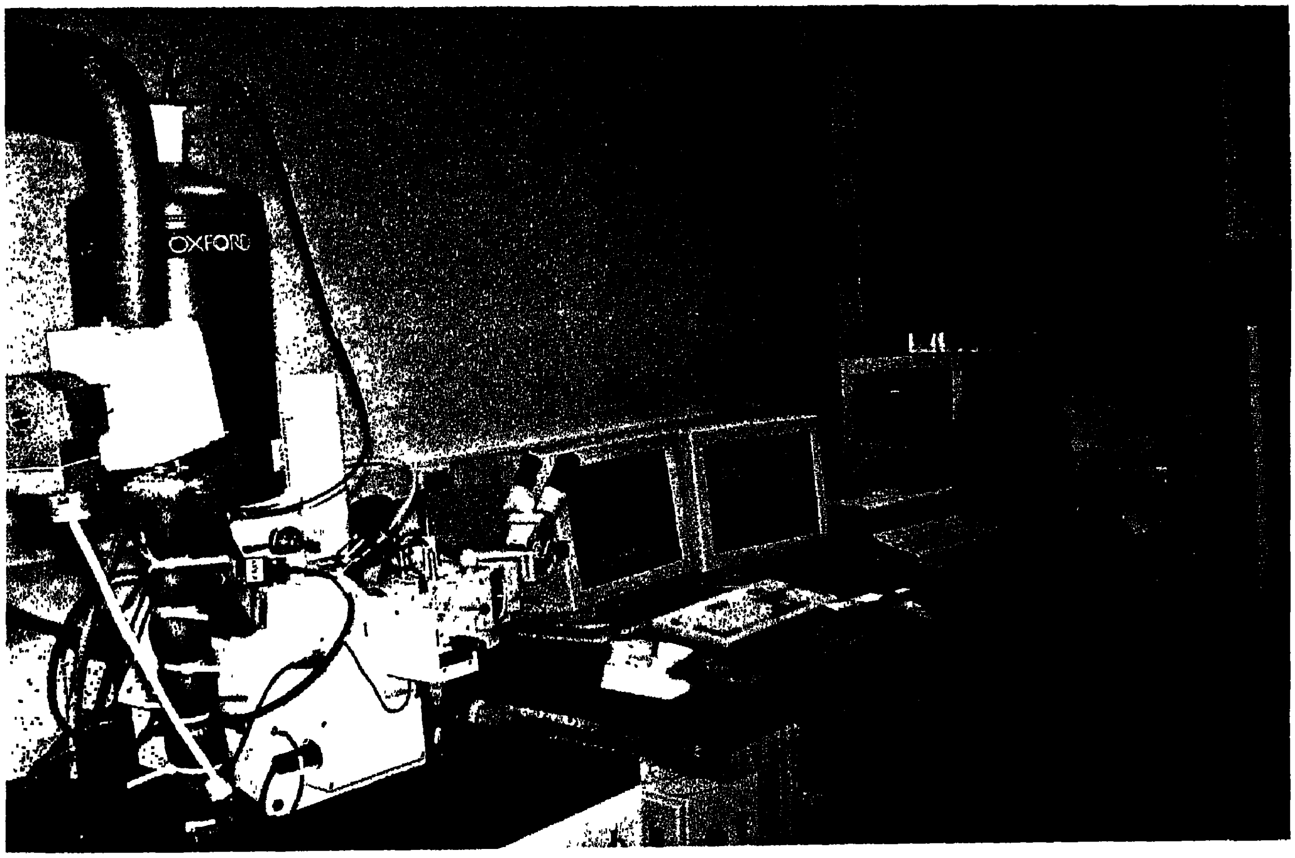
ANNEXURE- 5b

Scanning electron microscopy lab at INRA-Nancy, Champenoux, France



Abstract 1

There have been many reports suggesting the involvement of reactive oxygen species (ROS) including the superoxide anion ($O_2^- \bullet$) in salt stress. Here we report on direct evidence that treatments of cell suspension cultures of tobacco (*Nicotiana tabacum L.*; cell line, BY-2) with various salts of trivalent, divalent and monovalent metals stimulate the immediate production of $O_2^- \bullet$. Among the salts tested, $LaCl_3$ and $GdCl_3$ induced the greatest responses in $O_2^- \bullet$ production, while $CaCl_2$ and $MgCl_2$ showed moderate effects, and salts of monovalent metals such as KCl and NaCl induced much lower responses, indicating a strong relationship between the valence of metals and the level of $O_2^- \bullet$ production. As the valence of the added metals increased from monovalent to divalent and trivalent, the concentrations required for maximal response were lowered. Although $O_2^- \bullet$ production by NaCl and KCl required high concentrations associated with hyperosmolarity, the $O_2^- \bullet$ generation induced by NaCl and KCl was significantly greater than that induced simply by hyperosmolarity. Since an NADPH oxidase inhibitor, diphenyleneiodonium chloride, showed a strong inhibitory effect on trivalent and divalent cation-induced generation of $O_2^- \bullet$, it is likely that cation treatments activate the $O_2^- \bullet$ generating activity of NADPH oxidase.

Annexure 5 d

Abstract 2

Salts at high concentrations may cause oxidative damage to plant cells since many studies have indicated the involvement of reactive oxygen species in salt-stress response. Recently, we have demonstrated that treatments of a tobacco (*Nicotiana tabacum*) cell suspension culture with various salts results in an immediate burst of superoxide production via activation of NADPH oxidase by ions of alkali metals (Li^+, Na^+, K^+), alkali earth metals (Mg^{2+}, Ca^{2+}) or lanthanides (La^{3+}, Gd^{3+}). In this study, we tested the effect of extracellular supplementation of Zn^{2+} and Mn^{2+} on the cation-induced oxidative burst in tobacco cell suspension culture, measured with a superoxide-specific Cypridina luciferin-derived chemiluminescent reagent. Extracellular supplementation of Zn^{2+} and Mn^{2+} inhibited the generation of superoxide in response to addition of salts. Although both Zn^{2+} and Mn^{2+} inhibited the salt-induced generation of superoxide, the modes of inhibition by those ions seemed to be different since Mn^{2+} simply inhibited total production of superoxide while Zn^{2+} inhibited the early phase of superoxide production and induced the slow release of superoxide. The roles of Mn^{2+} and Zn^{2+} in the protection of plant cells from salt stress, as an effective superoxide scavenger and an effective inhibitor of plasma membrane-bound NADPH oxidase, respectively, are discussed.

III– PERSONNEL

A. INDIAN SIDE

Dr Alok Adholeya
Dr S Krishna Sundari
Dr Rama B Verma
Dr Atimanav Gaur
Mr Prasun Ray
Ms Pragati Tiwari

Name	Designation	Qualification	Date & Duration of appointment
Dr Krishna Sundari	Ph.D. Fellow	Ph.D.	2 ½ years 01.09.1999 – 30.03.2002
Dr Rama B. Verma	Ph.D. Fellow	Ph.D.	1 ½ year 01.04.2000 – 31.12.2001
Dr Atimanav Gaur	Ph.D. Fellow	Ph.D.	8 months 01.01.2002 – 31.08.2002
Mr Prasun Ray	Junior Research Fellow	M. Sc.	One year 01.09.2001- 31.08.2002
Ms Pragati Tiwari	Junior Research Fellow	M.Sc., M.Phil.	One year 01.09.2001- 31.08.2002

B. FRENCH SIDE

Frederic Lapeyrie, Dr, Directeur de Recherches
Tomonori Kawano, Dr, Post-Doc

Name	Designation	Qualification	Date & Duration of appointment
Dr Krishna Sundari	Research Associate	Ph.D.	One year 25.04.2000 – 24.04.2001
Mrs Anne Jambois	Ph.D. Student	M.Sc.	One year 01.01.2001 – 28.02.2002

IV– FINANCIAL ASPECTS

FUNDS APPROVED IN THE BUDGET RECEIVED FROM IFCPAR AND SPENT

A. INDIAN SIDE:

DETAILS	AMOUNT IN APPROVED BUDGET Rs.	AMOUNT RECEIVED Rs.	AMOUNT SPENT Rs.
SALARIES	4,76,400.00	3,55,840.00	3,90,640.00
RECURRING	10,00,000.00	9,37,070.00	10,56,872.00
EQUIPMENT	4,00,000.00	4,00,000.00	4,04,225.50
TOTAL	18,76,400.00	16,92,910.00	18,51,737.50

B. FRENCH SIDE:

DETAILS	AMOUNT IN APPROVED BUDGET Fr	AMOUNT RECEIVED Fr	AMOUNT SPENT Fr
SALARIES	3,50,000	3,50,000	3,46,913.06
RECURRING	1,50,000	1,50,000	1,53,086.94
EQUIPMENT	0	0	0
TOTAL	5,00,000	5,00,000	5,00,000.00

V– EQUIPMENT

Procurement of Equipment

A. INDIAN SIDE

NAME OF THE EQUIPMENT	VALUE Rs.	DATE OF RECEIPT
1. Benchtop centrifuge	Rs. 3,97,839.00	25th March, 2000

B. FRENCH SIDE

NAME OF THE EQUIPMENT	VALUE Fr	DATE OF RECEIPT

No equipment purchased

VI – EXCHANGE VISITS

A. FROM INDIA TO FRANCE

NAME OF THE SCIENTIST	DATE & DURATION
1. Dr Alok Adholeya	12th March – 27th March, 2001
2. Mr Prasun Ray	5th January – 19th February, 2002
3. Dr Alok Adholeya	18th June – 10th July, 2002

B. FROM FRANCE TO INDIA

NAME OF THE SCIENTIST	DATE & DURATION
1. Dr Fredric Lapeyrie	10th January – 22nd January, 2000
2. Dr Fredric Lapeyrie	7th April – 21st April, 2002
3. Dr Valerie Legue	9th June – 15th June, 2002

VII – PUBLICATIONS

(Enclosed in the part II - work report in Chapter – 9)

VIII – ACHIEVEMENTS

The major achievement from this project has been capacity building and mutual learning from diverse specialization within the ambit of mycorrhiza between two collaborator groups.

The selection and optimization of a few candidate mycorrhizal strains resulted in building confidence towards the application of these isolates. Many fundamental questions were answered concerning optimum conditions of growth, mechanisms and properties of heavy metal immobilization, root fungus signalling, host dependent variation of expression and most importantly, basis for selecting strains for a stressed environment. Additional work can provide the stronger base needed to select a 'super bug' carrying properties such as extensive colonization of ashes and high rate of heavy metal immobilization besides growth promotion in plants.

IX – ASSESSMENT

Please give your own assessment of the way the project was carried out. We would appreciate receiving your free and frank views. Please mention difficulties, if any, faced during the implementation of this project. You may also give any suggestions for improving the method of implementation, of this project in particular and all IFCPAR projects in general.

TERI – India

This project was a unique opportunity for us to learn many specialized scientific skills on ectomycorrhiza systems for which this group is well known.

Human resource development and capacity building were two major achievements resulted through exchange visits. We enjoyed this collaboration and professional discussions on the results obtained. The professional ties became stronger through interactions within the scope of the project and collaborations are continuing.

The efficient project management coupled and extraordinary cooperation rendered by the Indo-French Centre is highly appreciated.

Signature
(Indian Principal Collaborator)

Signature
(French Principal Collaborator)

IX – ASSESSMENT

Please give your own assessment of the way the project was carried out. We would appreciate receiving your free and frank views. Please mention difficulties, if any, faced during the implementation of this project. You may also give any suggestions for improving the method of implementation, of this project in particular and all IFCPAR projects in general.

Nancy - France

This project has been a very enjoyable part of international co-operation. It was a unique opportunity to collaborate on a long-term basis with an Indian group, to reach a high level of mutual understanding and respect.

Along these 3 years I have been learning a lot from my Indian colleague experience in degraded land rehabilitation. We got involved, as well, in deep scientific controversy, very stimulating and enriching. Presently we are still carrying work together; our collaboration will extend much further, behind the limits of this project.

I deeply appreciated IFCPAR very efficient project management, even though it is sometimes difficult to comply with strict procedures.

Signature	Signature
(Indian Principal Collaborator)	(French Principal Collaborator)